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Drinking Water Criteria Document for Carbon Tetrachloride (Final Draft)

Life Systems, Inc., Cleveland, OH

Prepared for

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The Office of Drinking Water (ODW), U.S. Environmental Protection Agency has prepared a Drinking Water Criteria Document on carbon tetrachloride. This Criteria Document is an extensive review of the following topics:

- Physical and chemical properties of carbon tetrachloride
- Toxicokinetics and human exposure to carbon tetrachloride
- Health Effects of carbon tetrachloride in humans and animals
- Mechanisms of toxicity of carbon tetrachloride
- Quantification of toxicological effects of carbon tetrachloride

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I. SUMMARY

Carbon tetrachloride (CCl_4) is a colorless liquid at ambient temperature. It is miscible with many organic solvents, but has limited solubility in water (0.8 g/L). Its high vapor pressure favors rapid volatilization from water to air. Carbon tetrachloride is produced commercially from the chlorination of methane, propane, ethane, propylene or carbon disulfide, and its major use is in the production of chlorofluorocarbons. Carbon tetrachloride that enters the environment appears to be relatively stable, enduring for many years.

Several reports suggest that CCl_4 is readily absorbed from the gastrointestinal tract. About 60% of an oral dose was absorbed by six hours, and up to 86% was absorbed by 24 hours. Absorption from the lung has been estimated at about 30%. Carbon tetrachloride appears to be distributed to all major organs following absorption, including liver, blood, brain, kidney and muscle, with particularly high concentrations in fat. Carbon tetrachloride reached maximal concentrations in most tissues at approximately two to four hours following intragastric administration.

Carbon tetrachloride metabolism occurs primarily in the liver. The first step is thought be formation of trichloromethyl radical bound to an iron atom in the cytochrome heme moiety. This trichloromethyl radical is postulated to undergo a variety of reactions, including hydrogen abstraction to form chloroform, dimerization to form hexachloroethane or addition to cellular molecules. Further metabolism of the heme-bound trichloromethyl radical is postulated to result in the eventual formation of carbonyl chloride (phosgene).

Carbon tetrachloride and its volatile metabolites are excreted primarily in exhaled air, with smaller quantities in the urine and feces. Elimination of orally ingested CCl_4 occurred with an estimated half-time of four to six hours and most of an oral dose was excreted within one to two days. No reports were located regarding tissue accumulation and retention of CCl_4 during chronic exposure.

Carbon tetrachloride is toxic to animals, with reported oral lethality values ranging from an LDL_0 of 1,000 mg/kg in dogs to an LD_{50} of 12,800 mg/kg in mice. The tissue most affected by CCl_4 is the liver. Using release of liver enzymes into serum and histological examination as endpoints, single oral doses of 40 mg CCl_4/kg did not produce adverse effects, while doses of 80 mg/kg or higher did. Numerous studies have reported that oral doses ranging from about 100 to 4,000 mg/kg produce fatty infiltration, loss of cytochrome P-450 and other enzymes, inhibition of protein synthesis and histological alterations in the liver. High doses frequently resulted in hepatocellular necrosis, but effects observed following lower doses were largely reversible.

Kidney and lung are also affected following oral exposure to CCl_4. Doses of about 4,000 mg/kg resulted in lesions of the renal proximal tubule and pulmonary Clara cells and endothelial cells. These changes also appeared to be reversible when damage was not too severe.

The effects of chronic exposure to CCl_4 are similar to the effects of acute exposure: the liver is the most sensitive tissue, showing fatty infil-

tration, release of liver enzymes, inhibition of cellular enzyme activities, inflammation and ultimately cell necrosis. Rats exposed to doses of 1 mg CCl_4/kg for 12 weeks did not show measurable adverse effects, while doses of 10 or 33 mg/kg produced enzyme release, centrilobular vacuolization and necrosis.

Carbon tetrachloride has not been shown to be teratogenic. Newborn animals appear to be less sensitive to CCl_4 than older animals. An intraperitoneal dose of 2,400 mg/kg has been reported to have adverse effects on testicular function.

No evidence of mutagenic activity for CCl_4 has been found in bacterial test systems or in cultured liver cells, although increased gene crossover and mitotic recombination were observed in yeast cells exposed to 3,200 to 5,200 mg CCl_4/L.

Carbon tetrachloride is carcinogenic in animals, producing mainly hepatic neoplasms. Doses of about 30 mg/kg/day or higher for six months or longer have been found to produce increased frequency of hepatomas, hepatocellular adenomas and hepatocellular carcinomas in mice, rats and hamsters.

The effects of CCl_4 exposure in humans are similar to effects seen in animals, with the liver, kidney and lungs being most sensitive. Single oral doses of 2.5 to 15 mL (57 to 343 mg/kg) usually were without external effect, although some individual adults suffered adverse effects (including death)

from ingestion of as little as 1.5 mL (34 mg/kg). Doses of 0.18 to 0.92 mL (29 to 150 mg/kg) may be fatal in children.

Inhalation exposure also results in renal and hepatic damage. No ill effects resulted from a three-hour exposure to 63 mg/m^3, but a 70-minute exposure to 2,309 mg/m^3 did produce some liver effects. Higher levels (1,500 mg/m^3) may produce severe poisoning and death.

No strong evidence was located that CCl_4 (either oral or inhaled) produces cancer in humans, although cases of cancer in exposed individuals have been reported.

No evidence was located to indicate that any human subpopulation is more sensitive to CCl_4 than is the general population. A number of reports presented evidence that individuals who chronically consume large amounts of alcohol are considerably more sensitive than individuals who consume little alcohol.

The metabolism of CCl_4 is thought to proceed through the formation of the trichloromethyl free radical, but the details of the subsequent reactions that produce cell injury remain obscure. Reaction of the trichloromethyl radical with proteins and/or radical-initiated lipid peroxidation are two mechanisms that may be involved.

Pre-exposure to certain compounds has been reported to significantly potentiate the hepatotoxicity of CCl_4. These potentiating agents include

ethanol, methanol, isopropanol, polybrominated biphenyls (PBBs), polychlorinated biphenyls (PCBs), phenobarbital and chlordecone.

A study in rats, using release of livers enzymes and histological examination of liver as end-points, identified an acute oral No-Observed-Adverse-Effect-Level (NOAEL) of 40 mg/kg, and this was used to calculate one-day Health Advisory values of 4 and 14 mg/L in children and adults, respectively. A similar study in rats identified a dose of 20 mg/kg/day (9 doses over 11 days) as a short-term Lowest-Observed-Adverse-Effect-Level (LOAEL), and this was used to calculate ten-day Health Advisory values of 0.16 and 0.57 mg/L in children and adults, respectively. These values are similar to guidelines proposed previously by NAS, but are somewhat higher than guidelines proposed previously by USEPA. A 12-week study in rats identified a NOAEL of 1 mg/kg/day, and this value was used to calculate an AADI of 0.025 mg/L (25 µg/L). No prior guideline based on non-carcinogenic effects following long-term exposure was located.

Several calculations have been performed to estimate the risk of cancer from CCl_4 exposure. At the 10^{-6} risk level, these estimates range from 0.0003 mg/L (0.3 µg/L) to 0.0045 mg/L (4.5 µg/L).

II. PHYSICAL AND CHEMICAL PROPERTIES

A. Physical and General Properties

Carbon tetrachloride, or tetrachloromethane, is a colorless liquid with a molecular weight of 153.8 and a boiling point of 76.5°C (Weast 1972). It is a relatively nonpolar compound that is miscible in alcohol, acetone and other organic solvents (Weast 1972), but is only minimally soluble in water (0.8 g/L at 25°C) (Johns 1976). The properties of CCl_4 are given in Table II-1. Several partition coefficients (K_D's) (a measure of the relative solubility of a substance at equilibrium between two media which are in mutual contact) for CCl_4, listed in Table II-2, illustrate that this chemical is lipophilic.

The properties of CCl_4 favor volatilization of the compound from water to air. Carbon tetrachloride has a high vapor pressure (115.2 mm Hg at 25°C) (Johns 1976). The water/air partition coefficient of CCl_4 at 20°C is 1.1 by volume and about 1,000 by weight (Johns 1976). The rapid vaporization predicted from these properties has been confirmed by Dilling et al. (1975), who reported a half-life of CCl_4 evaporation of 29 minutes from a dilute aqueous solution at about 25°C.

B. Manufacture and Use

Carbon tetrachloride is produced industrially by the chlorination of methane, propane, ethane, propylene or carbon disulfide (Rams et al. 1979).

Table II-1 Properties of Carbon Tetrachloride

Property	Value
Chemical Abstracts Registry No.	56-23-5
Chemical Abstracts Name	Tetrachloromethane
Synonyms	Carbona, carbon chloride, carbon tet, methane tetrachloride, perchloromethane, tetrachlorocarbon
Trade names	Benzinoform, Fasciolin, Flukoids, Freon 10, Halon 104, Necatorina, Nectorine, Tetrafinol, Tetraform, Tetrasol, Univerm, Vermostricid
Chemical formula	CCl_4
Molecular weight	153.8
Physical appearance	Colorless liquid
Boiling point	76.5°C
Melting point	-23°C
Density	d_4^{20} 1.594
Vapor pressure	115.2 mm Hg at 25°C
Solubility	
Water	0.8 g/L
Acetone	Miscible
Benzene	Miscible
Ethanol	Miscible
Ethyl ether	Miscible
Octanol	Miscible

Adapted from IARC (1979), Johns (1976) and Weast (1972).

Table II-2 Partition Coefficients for Carbon Tetrachloride

Media	K_D		
	20°C	25°C	37°C
Olive oil/air		142	361
Serum/air		6	
Blood/air	3.7-5.3		2.4
Water/air	1.1		0.25
Olive oil/water[a]			1,440
Olive oil/serum[b]		23	
Olive oil/blood[c]			150

The partition coefficient (K_D) is the ratio of the relative solubility of carbon tetrachloride at equilibrium between two media which are in mutual contact.

[a]Derived from: (olive oil/air)(/water/air).
[b]Derived from: (olive oil/air)/(serum/air).
[c]Derived from: (olive oil/air)/(blood/air).

Adapted from Morgan et al. (1972), Sato and Nakajima (1979), Powell (1945) and Johns (1976).

In 1980, 3.22×10^8 kg was synthesized (USITC 1981). Carbon tetrachloride is also produced indirectly during the production of compounds such as vinyl chloride and perchloroethylene (Rams et al. 1979).

The major use of CCl_4 is in the production of chlorofluorocarbons, which are used as refrigerants, foam-blowing agents and solvents. Carbon tetrachloride is also used in fumigants, as a solvent in metal cleaning and in manufacture of paints and plastics (Rams et al. 1979). It is being replaced in grain fumigation by other registered pesticides (USEPA 1980a), and its registration for use in fumigants is presently under review by USEPA (1980b).

C. Environmental Effect and Stability

Carbon tetrachloride present in the environment appears to be of anthropogenic origin (Singh et al. 1976). It can enter natural waters as a result of industrial and agricultural activities. Carbon tetrachloride may be carried to surface waters through run-off from agricultural, industrial or dumping sites and through industrial effluents. Industrial emissions may also contribute CCl_4 to the air, from which the compound may reach surface water through rainfall. Carbon tetrachloride may reach groundwater through leaching from solid waste sites.

The density of CCl_4 is 1.59 g/mL at 20°C (Weast 1972). Because its density is greater than the density of water, some CCl_4 from large spills into water might tend to settle before it is totally dispersed, emulsified or volatilized.

Once in the environment, CCl_4 is relatively stable. Its half-life for hydrolytic breakdown in water at pH 1.0 to 7.0 is estimated to be 70,000 years, but hydrolysis appears to be accelerated in the presence of metals such as iron and zinc (Johns 1976). The atmospheric lifetime of CCl_4 appears to be on the order of 30 to 100 years (Singh et al. 1976).

D. Summary

Carbon tetrachloride is a colorless liquid at ambient temperature. It is miscible with many organic solvents, but has limited solubility in water (0.8 g/L). Its high vapor pressure favors rapid volatilization from water to air. Carbon tetrachloride is produced commercially from the chlorination of methane, propane, ethane, propylene or carbon disulfide, and its major use is in the production of chlorofluorocarbons. Carbon tetrachloride that enters the environment appears to be relatively stable, enduring for many years.

III. TOXICOKINETICS

A. Absorption

No reliable study was located which directly measured the percent absorption of CCl_4 from the gastrointestinal tract after oral ingestion. However, a number of investigations have measured the extent of CCl_4 elimination in expired breath in rats. From these data, minimum estimates of absorption from the gastrointestinal tract can be deduced.

Paul and Rubinstein (1963) studied the absorption of ^{14}C-CCl_4 in rats following intraduodenal injection of 1 mL ^{14}C-CCl_4/kg (1,600 mg CCl_4/kg). The number of animals, sex, strain and body weight were not specified. Toluene-trapped radioactivity ($^{14}CCl_4$, confirmed by gas chromatography) and $^{14}CO_2$ in expired air were measured at 2, 4, 6, 12 and 18 hours after CCl_4 administration. The major substance in expired air was unchanged CCl_4, which increased steadily from 10% of the total dose at two hours to 78% at 12 hours and 85% at 18 hours. Radioactive metabolites in expired air, identified as chloroform and carbon dioxide, were less than 0.4% and 0.8%, respectively, of the total dose over the period studied. It can be concluded that at least 86% of the administered dose was absorbed from the gastrointestinal tract within 18 hours.

Seawright and McLean (1967) studied the absorption of CCl_4 in rats following intragastric administration of ^{14}C-CCl_4 (as a 50% solution in paraffin oil) at a dose of 2.5 mL CCl_4/kg (4,000 mg/kg). Toluene-trapped

radioactivity, assumed to be unaltered CCl_4 (no analytical analysis of this fraction was reported) and $^{14}CO_2$ from expired air was determined 2, 6 and 24 hours after treatment. No fewer than three male Wistar strain rats were used for each time interval. At 2, 6 and 24 hours the exhaled CCl_4 was 10%, 40% and 65%, respectively, of the total administered dose. A radioactive metabolite, identified as ^{14}C-CO_2, was no greater than 0.4% of the total dosage after 24 hours. It can be inferred that no less than 65% of the administered CCl_4 was absorbed from the gastrointestinal tract within 24 hours.

Absorption of CCl_4 from the gastrointestinal tract of rats was reported by Marchand et al. (1970). Male Sprague-Dawley strain rats, weighing 180 to 240 g, were given 2.0 mL undiluted ^{14}C-CCl_4/kg (3,200 mg CCl_4/kg) by intragastric intubation. The amount of toluene-trapped radioactivity in expired air was determined at 2, 4, 6 and 10 hours after gavage in six animals. Cumulative, toluene-trapped radioactivity, assumed to be unchanged ^{14}C-CCl_4, increased from 20% of the administered dose at two hours to 83% at ten hours. It can be deduced that at least 83% of the total dose was absorbed from the gastrointestinal tract within ten hours.

Reddrop et al. (1981) reported the absorption of CCl_4 from the gastrointestinal tract in rats. Male Tif RAI f (SPF) rats, weighing approximately 200 g, were given CCl_4 (in paraffin oil) by intragastric intubation at a dose of 13 mmol CCl_4/kg (2,000 mg CCl_4/kg). Samples of expired air from each of four animals were taken at intervals up to six hours from a flow-through

breathing apparatus containing a toluene trap. Electron-capture, gas chromatographic analysis of the toluene revealed that during the six hours following CCl_4 treatment, nearly 60% of the dose was expired unchanged. This indicates that no less than 60% of the administered dose was absorbed within six hours after treatment.

McCollister et al. (1951) investigated the absorption of CCl_4 via inhalation using rhesus monkeys. Three female monkeys inhaled ^{14}C-labeled CCl_4 (99.9% purity) at an average concentration of 290 mg/m^3 (46 ppm) for 139, 344 or 300 minutes, respectively. The authors calculated by difference between inhaled and exhaled air that the monkeys absorbed an average of 30.4% of the total amount of CCl_4 inhaled.

B. Distribution

Von Oettingen et al. (1950) measured the tissue distribution of CCl_4 in beagle dogs, each weighing about 10 kg, exposed to CCl_4 in air at 94,500 mg/m^3 (15,000 ppm) for 475 minutes. The dogs were sacrificed at the end of the exposure. Tissue and blood samples were taken and analyzed for CCl_4. The concentration of CCl_4 was 66 mg/100 g tissue in the brain, 50 mg/100 g in the heart, 36 mg/100 g in the liver and 34 mg/100 g in the blood. The concentration in fat was not determined. The investigators stated that the accumulation of CCl_4 in the brain was consistent with its high oil/water partition coefficient and resulted in its strong narcotic action.

McCollister et al. (1951) reported the tissue distribution of CCl_4 in a rhesus monkey exposed to 46 ppm (290 mg/m^3) of ^{14}C-CCl_4 for 300 minutes. The tissue distribution, as calculated from the ^{14}C radioactivity, is shown in Table III-1. The concentration of CCl_4 was greatest in fat, followed by the liver and bone marrow.

Fowler (1969) studied the distribution of CCl_4 in the tissues of rabbits dosed by stomach tube. Fifteen rabbits were given 1 mL CCl_4/kg (1,600 mg CCl_4/kg) as a 20% (v/v) solution in olive oil. Rabbits were sacrificed 6, 24 or 48 hours after treatment, and the tissues were analyzed for CCl_4 by a gas chromatograph equipped with an electron-capture detector. Six hours after CCl_4 was administered, the tissue concentrations were 787 ± 289 (mean ± SEM) mg/kg in fat, 96 ± 11 mg/kg in liver, 21 ± 12 mg/kg in muscle and 20 ± 13 mg/kg in kidney. By 48 hours, these concentrations had dropped to 45 ± 12 mg/kg in fat, 4 ± 0.1 mg/kg in liver and 0.5 ± 0.3 mg/kg in kidney and muscle. These data indicate that most of the dose of CCl_4 is eliminated from rabbit tissues within 48 hours.

Marchand et al. (1970) studied the tissue distribution of CCl_4 in rats following intragastric administration of ^{14}C-CCl_4. Male Sprague-Dawley strain rats, 180 to 240 g body weight, were given 2.0 mL undiluted ^{14}C-CCl_4/kg (3,200 mg ^{14}C-CCl_4/kg) by intragastric intubation. Blood and tissues were obtained 0.5, 1, 2, 5.5, 8 and 12 hours after gavage and extracted with toluene. No fewer than five animals were used for each time interval. Toluene-soluble radioactivity from liver, blood, muscle, brain and fat was

Table III-1 Tissue Distribution of ^{14}C-Carbon Tetrachloride Inhaled by a Rhesus Monkey[a]

Tissue	Carbon tetrachloride (mg/100 g of tissue)
Fat	2.46
Liver	0.94
Bone marrow	0.93
Blood	0.31
Brain	0.30
Kidney	0.23
Heart	0.14
Spleen	0.10
Muscle	0.06
Lung	0.04
Bone	0.04

[a]One female monkey was exposed to ^{14}C-CCl_4 (46 ppm) for 300 minutes.

Adapted from McCollister et al. (1951).

assumed to be unchanged ^{14}C-CCl_4. Maximum concentrations of CCl_4 appeared at two hours in all tissues examined except fat, where the highest level occurred 5.5 hours after treatment. Maximum mean concentrations of CCl_4 (per kilogram of tissue or per liter of blood) were 5,800 mg/kg in fat, 480 mg/kg in liver, 250 mg/kg in brain, 96 mg/kg in muscle and 90 mg/L blood.

Teschke et al. (1983) reported the distribution of CCl_4 in blood, liver and fat of rats following intragastric administration of CCl_4 in olive oil at a dose of 1.5 mL CCl_4/kg (2,400 mg CCl_4/kg). Blood and tissues were obtained from 30 female Wistar strain rats at 0, 3, 6, 12, 48 or 72 hours after gavage (five animals per interval). The concentration of CCl_4 was determined by gas chromatography utilizing a head-space sampling technique. In blood, the maximum concentration of CCl_4 (25 mg/mL) was noted at the earliest sampling time (three hours). Carbon tetrachloride levels remained maximal in the liver at 170 mg/kg tissue for three to six hours. By 48 hours, CCl_4 was no longer detected in blood or liver. In fat, the peak concentration of CCl_4 (39 mg/kg tissue) occurred at six hours after gavage and was no longer detected after 72 hours.

C. Metabolism

Chloroform was one of the first CCl_4 metabolites to be described (Butler 1961). Eight dogs were exposed to CCl_4 vapor via tracheal cannula at the rate of 8,000 mg/hour for three hours. At the cessation of exposure, the exhaled air from the dogs was collected and analyzed by both gas chromatography and

the Fujiwara reaction, a colorimetric procedure for the identification of chloroform. Chloroform was detected in the exhaled breath by both of these methods. The total amount of chloroform exhaled in two hours by each dog was estimated to be at 0.1 to 0.5 mg by gas chromatographic analysis. Tissue homogenates were also shown to metabolize CCl_4 to chloroform.

Evidence of metabolism to a free radical was suggested by studies showing hexachloroethane to be a CCl_4 metabolite (Bini et al. 1975). Wistar rats of about 200 g body weight were administered a single dose of 160 to 800 mg of CCl_4 (diluted in liquid paraffin) by gavage following a 24-hour fast. Five animals were used for each dosage level. The animals were sacrificed 15 minutes to 8 hours after treatment. A graph displaying CCl_4 concentrations in rat liver versus time showed levels of approximately 0.9 mg/g of tissue after 15 minutes and 1.7 mg/g after 120 minutes. Analysis of the gas chromatographic data showed the presence of chloroform at levels of 0.037 mg/g after 15 minutes; after four hours it had declined to 0.007 mg/g. Hexachloroethane was also present after four hours at levels of 0.005 mg/g. The authors explained the formation of both chloroform and hexachloroethane as CCl_4 metabolites by proposing that the trichloromethyl free radical was the primary metabolite of CCl_4.

Radioactive carbon dioxide was detected in the exhaled air of rhesus monkeys after a 344-minute exposure to ^{14}C-CCl_4 by inhalation (McCollister et al. 1951). The authors estimated that 4.4 mg (11%) of the total amount of radioactivity eliminated was excreted as carbon dioxide, with most of this occurring within seven days.

Shah et al. (1979) studied the metabolism of ^{14}C-CCl_4 by rat liver in vitro. Samples of liver homogenate equivalent to 0.167 g of tissue were incubated for 30 minutes at 37.5°C with 10 µmol of ^{14}C-labeled CCl_4, alone or with NADH or NADPH (or both). Radioactive carbon dioxide was detected by scintillation counting. The results are shown in Table III-2. The addition of NADPH or NADH appeared to result in substantial conversion of CCl_4 to carbon dioxide. Addition of both NADH and NADPH did not increase the conversion over that seen with NADPH alone.

Shah et al. (1979) tested for the possible formation of carbonyl chloride (phosgene) during hepatic CCl_4 metabolism by adding L-cysteine to the in vitro rat liver system described above. Carbonyl chloride and L-cysteine are known to react chemically to form a condensation product, 2-oxothiazolidine-4-carboxylic acid. The presence of this condensation product was confirmed by thin-layer chromatography and mass spectrometry. The authors inferred from the presence of 2-oxothiazolidine-4-carboxylic acid that carbonyl chloride was formed by the metabolism of CCl_4 by rat liver microsomes. The authors postulated a mechanism for biotransformation of CCl_4 which involved sequential oxidaton of CCl_4 while bound to a heme (see Figure III-1). Release of bound intermediates then could give rise to different metabolites at the site of release.

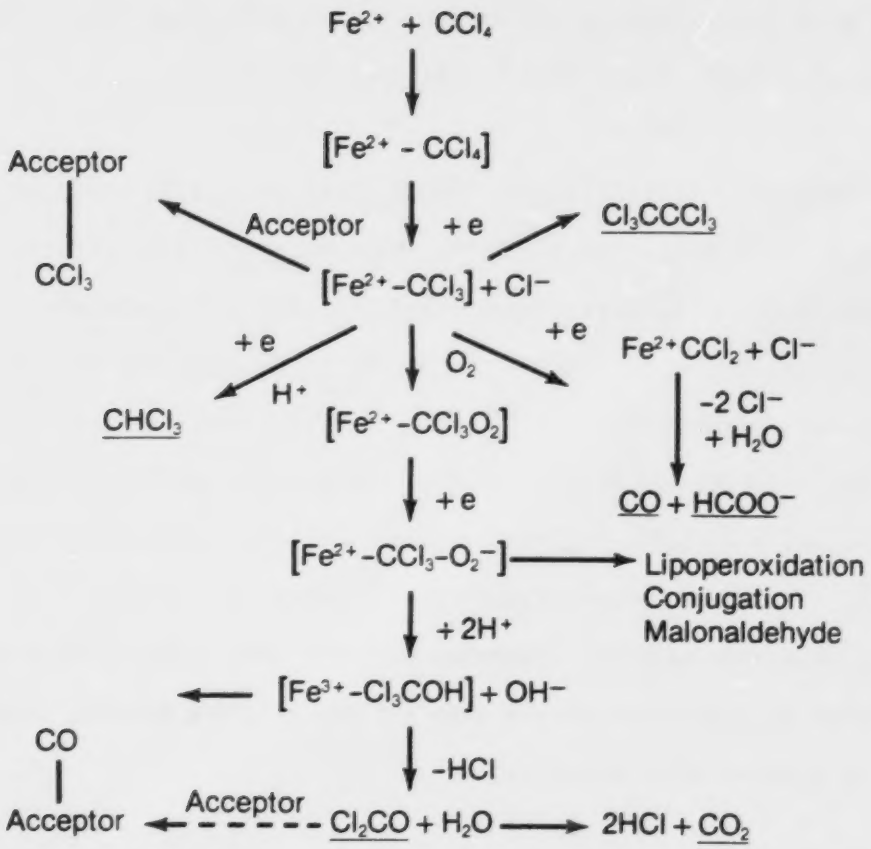
Fowler (1969) detected hexachloroethane and chloroform in the tissues of rabbits administered CCl_4 orally. A total of 15 rabbits were given CCl_4 as a 20% solution in olive oil at a dose of 1 mL/kg (1,600 mg/kg) and sacrificed in

Table III-2 Conversion of ^{14}C-Carbon Tetrachloride to ^{14}C-Carbon Dioxide by Rat Liver Homogenate

Nucleotide added	^{14}C-CO_2 (nmol/g liver)
None	27±5[a]
NADH	373±17
NADPH	464±33
NADH + NADPH	472±21

[a] Values are reported as mean ± S.E.M.

Adapted from Shah et al. (1979).



[a]Products identified as carbon tetrachloride metabolites are underlined. The electrons utilized in the reactions are assumed to come from NADH or NADPH via the flavoprotein cytochrome reductases. Ferro- and ferricytochromes are denoted by Fe^{2+} and Fe^{3+}, respectively.

Adapted from Shah et al. (1979).

Figure III-1 Pathways of Carbon Tetrachloride Metabolism[a]

groups of five at 6, 24 or 48 hours after exposure. Analysis of samples of fat, liver, kidney and muscle tissues by gas chromatography revealed the presence of chloroform and hexachloroethane (Table III-3). Fat contained the highest amounts of hexachloroethane at each sampling time, while high concentrations of chloroform appeared in both fat and liver.

Incorporation of label from ^{14}C-CCl_4 into liver lipids and proteins was reported by Rao and Recknagel (1969). Male Sprague-Dawley strain rats, weighing 143 to 212 g, were administered CCl_4 (as a 50% solution in mineral oil) at a dose of 2.5 mL ^{14}C-CCl_4/kg (4,000 mg ^{14}C-CCl_4/kg) by intragastric intubation. Incorporation of ^{14}C-CCl_4 into microsomal and mitochondrial lipids and proteins isolated from liver homogenates was observed at very early times following gavage. Incorporation of label into microsomal and mitochondrial lipids was maximal within five minutes and remained high for 90 minutes, the longest period studied. Proteins in these same subcellular fractions were not labeled as rapidly or to the same extent, reaching maximal levels between 30 and 90 minutes after poisoning.

D. Excretion

McCollister et al. (1951) studied the elimination of ^{14}C-labeled CCl_4 from rhesus monkeys exposed by inhalation for 344 minutes. The total radioactivity in the blood decreased 12% during the first ten minutes after exposure. Graphs of data from the analysis of blood samples obtained periodically for 10 to 12 days following exposure showed that the level of CCl_4 in

Table III-3 Chloroform and Hexachloroethane in Tissues of Rabbits Given Carbon Tetrachloride Orally[a]

Sample time	Tissue	$CHCl_3$ (μg/g tissues)	CCl_3CCl_3 (ng/g tissue)
6 hours	Fat	4.7±0.5	4.1±1.2
	Liver	4.9±1.5	1.6±0.5
	Kidney	1.4±0.6	0.7±0.2
	Muscle	0.1±0.1	0.3±0.2
24 hours	Fat	1.0±0.2	16.5±1.5
	Liver	1.0±0.4	4.2±1.8
	Kidney	0.4±0.2	2.2±1.1
	Muscle	0.1±0.1	0.5±0.2
48 hours	Fat	0.4±0.1	6.8±2.4
	Liver	0.8±0.2	1.0±0.3
	Kidney	0.2±0.0	trace
	Muscle	0.1±0.1	trace

[a] Values given as mean ± S.D. Carbon tetrachloride, 1,600 mg/kg was administered by stomach tube as a 20% solution in olive oil (v/v). Five animals used for each sample time.

Adapted from Fowler (1969).

the blood decreased exponentially with time. At ten days, the level of CCl_4 in blood was approximately 0.007 mg/100 g. The authors estimated that 21% of the total amount of CCl_4 absorbed was eliminated in expired air during the first 18 hours. By extrapolation of these data, the authors concluded that after 1,800 hours (75 days), approximately 51% of the CCl_4 initially absorbed would be eliminated in exhaled breath as CCl_4 and carbon dioxide. Analysis of urine showed 1.7 mg/100 g shortly after exposure, and this level fell to low values by five days. Fecal excretion reached maximum at two days after exposure (peak value = 1.1 mg/100 g), and this also fell to low levels within five to six days. The authors interpreted these findings as indicating that the other 49% of absorbed CCl_4 was excreted mainly in the urine and feces as CCl_4 or metabolites. However, since the total volume of urine and feces eliminated over the duration of the experiment was not measured, and other tissues (particularly fat) were not monitored for radioactivity, the amount of CCl_4 remaining in body tissues cannot be determined.

Similar results by Paul and Rubinstein (1963), Seawright and McLean (1967), Marchand et al. (1970) and Reddrop et al. (1981) indicate that most orally administered CCl_4 is eliminated unchanged via exhaled breath within several hours after treatment.

E. Bioaccumulation and Retention

No studies were located which provide data on tissue or body levels of CCl_4 following long-term oral exposure. Two reports which document the rapid

elimination of a single, oral dose from liver and other tissues are summarized below.

The concentration of CCl_4 in the liver of rats as a function of time following intragastric administration of CCl_4 was reported by Recknagel and Litteria (1960). A dose of 2.5 mL/kg (4,000 mg/kg) was administered as a 50% solution in mineral oil by gastric intubation to 39 male rats (body weight and strain not specified) which had been fasted for 10 to 12 hours. The concentration of CCl_4 in liver was determined as the Fujiwara chromogen utilizing a microdiffusion cell technique. Liver samples were obtained at several intervals between 0.5 and 30 hours after gavage. Maximum content of CCl_4 in the liver, 800 to 1500 µg/g liver, was observed at 1.5 hours. By 20 to 30 hours, the content had fallen to less than 200 µg CCl_4/g liver.

Marchand et al. (1970) studied the accumulation and clearance of CCl_4 in several tissues of rats, following intragastric administration of CCl_4. Male Sprague-Dawley strain rats weighing 180 to 210 g, were given 2.0 mL undiluted ^{14}C-CCl_4/kg (3,200 mg ^{14}C-CCl_4/kg) by intragastric intubation. Liver, blood, muscle, brain and fat samples were extracted with toluene at several intervals from 0.5 to 12 hours after gavage. Toluene-soluble radioactivity, assumed to be unchanged ^{14}C-CCl_4, was measured from tissues of no fewer than five animals per time point. Initial clearance rates (estimated from graphical data) indicated half-time ^{14}C-CCl_4 retention values of 4.5 to 6 hours for each tissue examined. However, since measurements of ^{14}C-CCl_4 were made over only 12 hours with relatively few time points, this derivation of clearance half-times cannot be considered highly accurate.

F. Summary

Several reports suggest that CCl_4 is readily absorbed from the gastrointestinal tract. About 60% of an oral dose was absorbed by six hours, and up to 86% was absorbed by 24 hours. Absorption from the lung has been estimated to be about 30%.

Carbon tetrachloride appears to be distributed to all major organs following absorption. Carbon tetrachloride has been found in fat, liver, blood, brain, kidney and muscle, with particularly high concentrations in fat. Carbon tetrachloride reached maximal concentrations in most tissues at approximately two to four hours following intragastric administration.

Carbon tetrachloride metabolism occurs primarily in the liver. The first step is thought be formation of trichloromethyl radical bound to an iron atom in the cytochrome heme moiety. This trichloromethyl radical has been postulated to undergo a variety of reactions, including hydrogen abstraction to form chloroform, dimerization to form hexachloroethane or addition to cellular molecules. Further metabolism of the eme-bound trichloromethyl radical has been postulated to result in the eventual formation of carbonyl chloride (phosgene).

Carbon tetrachloride and its volatile metabolites are excreted primarily in exhaled air and also in the urine and feces. Elimination of orally ingested CCl_4 occurred with an estimated half-time of four to six hours, and most of an

oral dose was excreted within one to two days. No reports were located regarding the tissue accumulation and retention of CCl_4 during chronic exposure.

IV. HUMAN EXPOSURE

This section will be provided by the Science and Technology Branch, ODW.

V. HEALTH EFFECTS IN ANIMALS

A. Acute Toxicity

1. Lethality

The acute lethality of orally administered CCl_4 in several species is summarized in Table V-1. Reported values range from an LDL_0 of 1,000 mg/kg in dogs to an LD_{50} of 12,800 mg/kg in mice.

2. Effects on Liver

A series of experiments to determine the effects of single CCl_4 exposures on rats were performed by Murphy and Malley (1969). Adult male Holtzman rats (250 to 350 g) were administered various doses of undiluted CCl_4 by gavage. Control animals were administered equal volumes of water. At 2 to 20 hours after treatment, animals were sacrificed and liver enzyme activities and liver weights were measured. The results are shown in Table V-2. The animals receiving CCl_4 at 1,600 mg/kg were sacrificed 20 hours after treatment, and the livers were examined histopathologically. The examination showed extensive fatty infiltration, inflammation and some centrilobular necrosis. The liver-to-body weight ratios were also increased.

Murphy and Malley (1969) also determined the effect of single exposures to CCl_4 on the activities of the corticosterone-inducible liver enzymes

Table V-1 Acute Oral Lethality of Carbon Tetrachloride in Animals

Species	Dose (mg/kg)	Lethality
Dog	1,000	LDL_0
Rat	2,800	LD_{50}
Rabbit	6,300	LD_{50}
Mouse	12,800	LD_{50}

Adapted from NIOSH (1980).

Table V-2 Effects of Oral Carbon Tetrachloride on Liver Weight and Liver and Plasma Enzyme Activities[a] in Male Rats

Dose (mg/kg)	Time (hours)	Number of animals	Plasma AKT[b]	Liver Enzymes			Liver weight (g/100 g bw)
				AKT[b]	TKT[c]	AP[d]	
0	--	7	2.6±0.3	2,350±182	14.9±1.0	14.9±1.0	2.75±0.06
3,200	2	4	2.1±0.2	--	14.5±1.0	14.5±1.0	2.94±0.04
3,200	5	5	13.2±3.4	1,813±331	14.2±1.3	14.2±1/3	3.26±0.10
3,200	20	5	35.2±4.2	1,174±559	34.6±5.8	34.6±5.8	4.36±0.06
2,400	20	4	35.2±3.4	1,585±148	37.1±1.6	37.1±1.6	3.95±0.07
1,600	20	5	18.1±4.3	1,596±194	33.3±3.6	33.3±3.6	3.90±0.05
800	20	4	9.3±2.3	2,120±182	29.1±6.5	29.1±6.5	3.47±0.07

[a]Micromoles of product formed per gram of fresh liver or milliliter of plasma per hour. Values given as mean ± SE.
[b]Alanine-α-ketoglutarate transaminase.
[c]Tyrosine-α-ketoglutarate transaminase.
[d]Alkaline phosphatase.

Adapted from Murphy and Malley (1969).

tryptophan pyrrolase and tyrosine-α-ketoglutarate transaminase. Groups of rats (four to six in each group, eight untreated controls) were treated with undiluted CCl_4 (0, 400, 800 or 1,600 mg/kg) and sacrificed five hours after treatment. Enzyme levels were found to increase roughly in proportion to the dose.

Recknagel and Ghoshal (1966) found an increase in hepatic triglyceride content after intragastric administration of CCl_4. Following a 16-hour fast, rats weighing 104 to 184 g were given 0, 1, 2.5 or 6.25 µL CCl_4/100 g (0, 16, 40 or 100 mg CCl_4/kg, given as a mineral oil solution in a total volume of 5 mL/kg) by gastric intubation under light ether anesthesia. Control animals received 5 mL mineral oil/kg. The sex, strain and number of animals used were not specified. Liver triglyceride content was determined six hours after treatment. The control level of triglyceride was 14.3 ± 2.31 mg/liver/100 g body weight. Triglyceride contents of rats given 16, 40 or 100 mg CCl_4/kg were 32.5 ± 6.95, 41.8 ± 4.46 or 51.5 ± 4.55 mg/liver/100 g body weight, respectively. The lowest dose, 16 mg CCl_4/kg, produced a 2.3-fold increase in hepatic triglyceride within six hours ($P < 0.05$ compared to control), and larger increases were produced by the higher doses.

Reynolds and Yee (1968) studied the effect of CCl_4 on glucose-6-phosphatase and calcium influx in the liver. Following a 16-hour fast, male rats, 150 to 300 g body weight, were given 0 to 2,600 µmol CCl_4/100 g (0 to 4,000 mg CCl_4/kg) intragastrically as a mineral oil solution. Control animals received mineral oil. The strain and number of animals used were not

reported. Glucose-6-phosphatase activity and calcium content were determined by histochemical techniques in livers one hour after treatment. The degree of hepatic necrosis was evaluated morphologically 24 hours after treatment. Overall, decreasing doses of CCl_4 decreased the extent of glucose-6-phosphatase suppression, stainable calcium and centrilobular necrosis. However, CCl_4 at the lowest dose (2.6 µmol CCl_4/100 g (4.0 mg CCl_4/kg)) still resulted in a decrease in centrilobular staining intensity specific for glucose-6-phosphatase activity. At 26 µmol CCl_4/100 g (40 mg CCl_4/kg), a transient mid-zonal calcium influx was observed. Centrilobular necrosis was noted 24 hours after doses of 83 µmol CCl_4/100 g (130 mg CCl_4/kg) or higher.

Alpers and Isselbacher (1968) studied the effect of CCl_4 on liver protein synthesis in rats. Carbon tetrachloride, 0.25 mL/100 g (4,000 mg/kg), was administered as a 50% solution in mineral oil by gastric intubation to rats weighing 160 to 180 g (strain and number of animals not specified). Protein synthesis by isolated hepatic polyribosomes was depressed 32% and 55% by 10 and 30 minutes after intubation, respectively. Breakdown of hepatic polyribosomes was significant (no P value given) in poisoned rats during this interval.

Lipoperoxidative decomposition of liver lipids following intragastric administration of CCl_4 (as a 50% solution in mineral oil) was reported by Rao and Recknagel (1968). Three male Sprague-Dawley rats, weighing 103 to 193 g, were given an intragastric dose of 0.25 mL CCl_4/100 g (4,000 mg CCl_4/kg), and the extent of hepatic lipid peroxidation was determined as diene conjugation absorption at 235 nm of extracted lipids. Three untreated rats served as

controls. The onset of microsomal lipid peroxidation was more than half maximal after 5 minutes and maximal at 30 minutes. Mitochondrial lipid peroxidation was also noted at 15 and 30 minutes, but to a lesser extent.

Glende (1972) reported the effect of oral administration of CCl_4 on liver cytochrome P-450 content. An intragastric dose of 25 µL CCl_4/100 g (400 mg CCl_4/kg) was given in mineral oil to male Sprague-Dawley rats weighing 200 to 300 g. Hepatic microsomal cytochrome P-450 content and drug metabolizing activity were determined at six time points after treatment (30 control and 30 experimental animals, five per interval). Cytochrome P-450 was depressed to 68% of control at three hours, the earliest period examined; the maximal decrease (to 35% of control) was seen at 24 hours. Cytochrome P-450 levels remained low for four days following CCl_4 gavage, but were returning toward normal values by day seven. Aminopyrine demethylase activity paralleled the loss and recovery of cytochrome P-450. During the period of maximum CCl_4-induced depression of cytochrome P-450, one to four days after gavage, rats were completely resistant to an otherwise lethal dose of CCl_4.

Ugazio et al. (1973) studied liver weight and neutral lipid content following intragastric administration of CCl_4. Male Sprague-Dawley rats were given CCl_4 in mineral oil by stomach tube at doses of 6.25 to 400 µL CCl_4/100 g (100 to 6,400 mg CCl_4/kg). The size and number of animals used were not specified. Liver weights and neutral lipid content were determined at 0, 6, 12, 24, 48 and 72 hours after gavage. Liver weight increased from 3.5 g/100 g (control) to 5.7 g/100 g in proportion to dosage within 24 hours to 48 hours.

Neutral lipid content increased in a similar manner, with the largest dose (6,400 mg CCl_4/kg) inducing a 25-fold rise. The lowest dose (100 mg CCl_4/kg) produced a four-fold increase in neutral lipid content within 12 hours after treatment.

Lowrey et al. (1981) studied calcium sequestration in liver microsomes from rats treated with CCl_4. Male Sprague-Dawley rats, weighing 150 to 250 g, were administered 0.025 to 5.0 mL CCl_4/kg (40 to 8,000 mg CCl_4/kg) by stomach tube. The high doses (1.0 to 5.0 mL/kg) were given undiluted, and the lower doses were given as mineral oil solutions. Hepatic microsomal calcium sequestration was measured five minutes after treatment, using three to six animals per dose and ten control animals. Calcium sequestration was reduced 13% at the lowest dose of 40 mg CCl_4/kg, 20% at 400 mg CCl_4/kg and approximately 70% at the higher doses of 1,600 to 8,000 mg CCl_4/kg. In a related study using 40 control and 30 experimental rats, the time course of CCl_4-induced loss of microsomal calcium sequestration was investigated. At a dose of 4,000 mg CCl_4/kg, calcium sequestration was reduced to 70% of control values within three minutes and to approximately 15% of control values by ten minutes. The authors showed that this inhibition of hepatic microsomal calcium sequestration coincided closely, both in extent and in time, with the CCl_4-induced liver microsomal lipid peroxidation as reported by Rao and Recknagel (1968).

Reversibility of the effect of acute CCl_4 administration on serum activity of liver enzymes in rats was studied by Drotman and Lawhorn (1978).

Groups of four male Cox rats were administered CCl_4 intraperitoneally at 60, 120, 240 or 480 mg/kg in a total volume of 1 mL of corn oil and exsanguinated at 6 to 168 hours after treatment. Serum activities were determined for the enzymes sorbitol dehydrogenase (SDH), ornithine carbamyl transferase (OCT), aspartate aminotransferase (AST) and isocitric dehydrogenase (ICDH). Liver samples were taken from each animal and examined for histopathological changes. The results of the enzyme analyses and histopathology are tabulated in Table V-3. Recovery from injury, judged by histological examination and serum enzyme assay, was observed at all doses of CCl_4 administered. Reversibility was evident 36 hours after doses of 60 mg CCl_4/kg and by 96 hours after doses of 120 to 480 mg CCl_4/kg. The OCT activities in serum showed the best correlation with liver histopathology in time of appearance as well as extent of damage. The authors concluded that serum OCT levels are a sensitive indicator of liver damage.

Effects of acute exposure to CCl_4 were also reported by Korsrud et al. (1972). Male Wistar rats (280 to 400 g, eight or nine animals per treatment group) were administered single oral doses of CCl_4 (0 to 4,000 mg/kg) in corn oil (5 mL/kg). The rats were fasted for six hours before treatment and for 18 hours afterward and then sacrificed. Assays included liver weight and fat content, serum urea and arginine levels and levels of nine serum enzymes originating mainly from the liver. At 20 mg/kg, there was minimal evidence of morphological changes in the liver. Higher doses resulted in some loss of basophilic stippling, a few swollen cells and occasional single cell necrosis. At a dose of 40 mg/kg, liver fat, liver weight, serum urea and levels of five

Table V-3 Effects of Intraperitoneal Administration of Carbon Tetrachloride on Liver Histopathology and Serum Enzyme Levels in Rats

CCl_4 (mg/kg)	Hours after dose	Histology[b]	Serum enzyme[a] concentrations relative to pretreatment levels			
			OCT	SDH	AST	ICDH
60	0	0	1.0	1.0	1.0	1.0
	6	2	9.6[c]	9.6[c]	2.0	1.4
	12	1	8.2[c]	4.4[c]	2.5[c]	1.1
	24	1	5.7[c]	1.7	2.0	1.3
	36	0	1.0	1.0	1.0	1.0
120	0	0	1.0	1.0	1.0	1.0
	24	3	14.0[c]	7.2[c]	2.1[c]	1.1
	48	2	7.4[c]	1.0	1.0	1.0
	96	1	1.8	1.7	1.3	1.3
	168	0	1.0	1.0	1.0	1.0
240	0	0	1.0	1.0	1.0	1.0
	24	3	31.0[c]	17.4[c]	5.8[c]	7.2[c]
	48	4	180.0[c]	43.3[c]	17.0[c]	7.4
	96	1	6.6[c]	4.7	3.6[c]	2.0
	168	1	1.1	1.0	1.9	2.0
480	0	0	1.0	1.0	1.0	1.0
	24	3	28.4[c]	90.0[c]	6.1[c]	5.4[c]
	48	4	465.5[c]	163.5[c]	18.4[c]	50.4[c]
	96	1	1.0	8.4[c]	1.8[c]	1.0
	168	0	1.0	1.4	1.0	2.1

[a] OCT, ornithine carbamyl transferase; SDH, sorbitol dehydrogenase; AST, aspartate aminotransferase; ICDH, isocitric dehydrogenase.

[b] 0 = No observable changes.
1 = Minimal changes. Large central vein, swelling of hepatocyte, etc.
2 = Mild degenerative change. Loss of cord arrangement.
3 = Moderate degenerative change. Pale cytoplasm, spindle cell.
4 = Marked degenerative change. Centrilobular fatty degeneration.

[c] Significantly different from zero time as determined by one-way analysis of variance of the log-transformed data ($P<0.01$).

Adpated from Drotman and Lawhorn (1978).

of the nine liver enzymes were increased, while serum arginine decreased. At higher doses, the remaining four enzyme levels were also elevated.

Bruckner et al. (1984) studied the acute oral toxicity of CCl_4 in rats. Two groups of male Sprague-Dawley strain rats, weighing 200 to 250 g or 300 to 350 g, were given (by gavage) single or multiple doses of CCl_4 (in corn oil) ranging from 0 to 160 mg CCl_4/kg. Serum enzyme activities one to eleven days after initiation of treatment are given in Table V-4 and Table V-5. Single doses of 20 or 40 mg CCl_4/kg had no toxic effect within 24 hours, but 80 mg CCl_4/kg produced significant increases in some enzyme levels as well as mild hepatic centrilobular vacuolization. Serum enzyme activities and histopathological evaluation over the 11-day period indicated progressively severe liver injury at each dose level.

Alumot et al. (1976) reported the effects of subchronic exposure to feed that had been fumigated with CCl_4. Groups of six weaning rats (four weeks old) were fed a diet containing CCl_4 at 150, 275 or 520 mg/kg of feed for five weeks (females) or six weeks (males). The fumigated feed was stored in airtight containers; CCl_4 loss during the storage period of seven to ten days was determined to be 5%. The animals were allowed access to the feed only at set time intervals to minimize loss of CCl_4 by volatilization. The authors calculated that the amount of CCl_4 remaining in the consumed feed was 60% to 70% of the amount initially present; the total decrease reflected amounts lost during storage and after removal from storage to feeding troughs. From these data and the weights of the animals, the authors calculated that 275 mg/kg of

Table V-4 Serum Enzyme Activities Following Oral Administration of Carbon Tetrachloride to Rats

Day of sacrifice after start of study	CCl_4 dosage (mg/kg)[a]		Serum enzyme activities[b]			BUN[c]
	Daily	Total	OCT[d]	SDH[e]	GPT[f]	(mg/dL)
1 (single dose)	0	0	101±39	4±1.4	12±5	17.8±0.7
	20	20	24±11	7±0.4	10±2	16.6±0.9
	80	80	112±18	43±14.0[g]	40±9[g]	15.4±0.9
	160	160	7,056±3,193[g]	204±17.1[g,h]	385±82[g,h]	9.2±0.6[g]
4 (4 doses)	0	0	78±24	3±0.4	22±3	N.D.[i]
	20	80	98±31	20±3.5[g]	33±2[g]	N.D.
	80	320	154±10[g]	82±15.3[g]	55±6[g]	N.D.
	160	640	179±50[g]	149±44.8[g,h]	89±6[g,h]	22.4±1.9
11 (9 doses)	0	0	68±12	3±0.2	10±1	N.D.
	20	180	102±24[g]	42±5.0[g]	48±7[g]	N.D.
	80	720	8,345±2,610[g]	217±50.3[g]	775±245[g]	N.D.
	160	1,440	588±180[g]	251±73.0[g,h]	1,156±429[g,h]	22.4±1.0

[a] Groups of five rats were dosed orally once daily for five days, rested for two days and dosed once daily for four additional days.
[b] Values are expressed as mean ± S.E.
[c] Blood urea nitrogen.
[d] Ornithine-carbamyl transferase activity, nmol CO_2/mL.
[e] Sorbitol dehydrogenase activity, I.U./mL.
[f] Glutamic-pyruvic transaminase activity, I.U./mL.
[g] Significantly different from control ($P<0.05$, paired t test).
[h] Significant increase with increasing dose ($P<0.05$, analysis of variance).
[i] Not determined.

Table V-5 Serum Enzyme Activities Following Oral Administration of Carbon Tetrachloride to Rats

Day of sacrifice after start of study	CCl_4 dosage (mg/kg)[a]		Serum enzyme activities[b]			BUN[c]
	Daily	Total	OCT[d]	SDH[e]	GPT[f]	(mg/dL)
1 (single dose)	0	0	N.D.[g]	4±0.7	12±2	14.6±0.8
	20	20	N.D.	4±0.6	15±2	19.0±1.4
	40	40	N.D.	7±1.8	20±3	19.6±1.0
	80	80	N.D.	9±2.9	25±4[h,i]	20.2±1.1[h]
4 (4 daily doses)	0	0	158±62	4±0.9	20±5	19.8±0.6
	20	80	163±30	10±3.9	26±4	19.8±0.4
	40	160	296±25	51±13.3[h]	55±14[h]	21.0±1.8
	80	320	260±75	62±3.0[h,i]	63±16[h]	17.7±1.1
11 (9 daily doses)	0	0	92±21	5±0.2	20±2	17.8±1.0
	20	180	167±17[h]	78±18.0[h]	44±9[h]	19.0±1.8
	40	360	372±72[h]	155±17.1[h]	351±110[h]	22.2±2.5
	80	720	737±147[h,i]	241±13.2[h,i]	469±66[h,i]	24.6±1.4[h]

[a]Groups of five rats were dosed orally once daily for five days, rested for two days and dosed once daily for four additional days.
[b]Values are expressed as mean ± S.E.
[c]Blood urea nitrogen.
[d]Ornithine-carbamyl transferase activity, nmol CO_2/mL.
[e]Sorbitol dehydrogenase activity, I.U./mL.
[f]Glutamic-pyruvic transaminase activity, I.U./mL.
[g]Not determined.
[h]Significantly different from control ($P<0.05$, paired t test).
[i]Significant increase with increasing dose ($P<0.05$, analysis of variance).

Adapted from Bruckner et al. (1984).

feed represented a daily dose of 40 mg/kg body weight. (By assuming that all parameters were the same and that the delivered dose was proportional to the concentration in feed, diets of 150 and 520 mg/kg of feed were calculated to represent daily doses of 22 and 76 mg/kg body weight, respectively.) At the end of the experiment, the animals were weighed and sacrificed. Of the three doses, only the highest, 76 mg/kg (520 mg/kg of feed), caused significantly depressed weight gain in males. Weight gain in females appeared to be unaffected by all doses. Total lipid and triglyceride levels in the liver were significantly higher in both male and female animals fed CCl_4 at 40 and 76 mg/kg body weight than in controls or animals fed 22 mg/kg body weight. Levels of liver phospholipids (measured in females) were not affected at any dose. Of the three doses used in this experiment, the lowest (22 mg/kg body weight) failed to produce effects on the measured parameters.

3. Effects on Kidney

The effect of oral CCl_4 administration on the structure and function of rat kidney was reported by Striker et al. (1968). Male Sprague-Dawley rats were given an intragastric dose of 0.25 mL CCl_4/100 g (4,000 mg CCl_4/kg). Morphological and chemical studies of the kidneys were made at intervals from 2 to 120 hours after treatment. Carbon tetrachloride-induced renal lesions were limited to the proximal tubule. As early as two hours after exposure, mitochondria were swollen, and cell swelling was observed at 12 hours. At this time, proliferation of smooth-surfaced endoplasmic reticulum was evident. These structural changes returned to normal by five days after CCl_4 exposure.

During the period of morphologic changes, there was also a significant decrease in water and sodium ion conservation in the poisoned animals.

Plaa and Larson (1965) studied the effect of CCl_4 on renal function in male Swiss mice. Animals (18 to 30 g) were given an intraperitoneal injection of CCl_4 (1,600 to 6,400 mg/kg) dissolved in corn oil. The animals were then hydrated with tap water (50 mL/kg) by gavage and placed on a urinary collection unit for two hours. Renal function was assessed by measuring urinary excretion of phenolsulfonephthalein (PSP), protein and glucose. At a dose of 3,300 mg/kg only minimal renal dysfunction was observed after 96 hours, although histologic examination of kidney sections from five mice that had been administered this dose showed necrosis of proximal convoluted tubules (n=1) and swelling of the tubules (n=4). Even the dose of 6,400 mg/kg (which was lethal to most animals) did not produce significant renal dysfunction in survivors.

Klaassen and Plaa (1967) studied the effect of CCl_4 exposure on kidney function in dogs. Five dogs were injected intraperitoneally with CCl_4 (in corn oil) at doses of 2,200 or 2,900 mg/kg, and the 24-hour excretion rate of PSP was determined. Control dogs were used to determine a normal range for PSP excretion. Urinary PSP excretion of less than 39% of control values was considered indicative of renal dysfunction. None of the dogs treated with CCl_4 exhibited decreased PSP excretion, although histological examination of the kidneys from the treated dogs revealed evidence of change (Bowman's capsules appeared dilated with some contraction of glomerular tufts and calcification of a small number of tubules in the medulla).

4. Effects on Lung

Boyd et al. (1980) investigated the effect of ingestion of CCl_4 on pulmonary Clara cells. Swiss mice were intubated with CCl_4 (4,000 mg/kg) in a 50% sesame oil solution and sacrificed 16 hours after treatment. The lungs were removed and examined by electron microscopy. Clara cells exhibited massive dilation of vesicles of smooth endoplasmic reticulum, increased mitochondrial staining density, ribosomal disaggregation, nuclear condensation and occasional cellular necrosis. Oral doses of less than 1,600 mg/kg did not result in any pulmonary lesions visible by light microscopy. Doses of 2,400 to 4,800 mg/kg produced Clara cell lesions similar to those previously described. The extent of damage was proportional to the dose administered. The time course of the Clara cell damage caused by ingestion of CCl_4 (4,000 mg/kg) was evaluated by light microscopy of pulmonary tissue at 12, 24, 36, 48, 96 and 168 hours after exposure. The lesions were present at 12 hours, maximal at 24 hours and less intense at 36 hours. By 48 hours, the lesions were seen infrequently, and at 96 and 168 hours the pulmonary bronchioles appeared normal.

Boyd et al. (1980) also observed lesions of the Clara cells in the lungs of male Sprague-Dawley rats treated orally with CCl_4. The CCl_4 was administered by gavage at doses of 3,800, 5,100 or 7,200 mg/kg, given as a 50% solution in sesame oil. Control animals received sesame oil only. Clara cell lesions occurred at the two highest doses. The authors stated that the lesions were less pronounced than those in mice exposed to comparable amounts of CCl_4.

Gould and Smuckler (1971) investigated structural alterations in rat lungs following CCl_4 ingestion. Male Sprague-Dawley rats (200 to 250 g) were fasted 16 hours prior to administration of CCl_4 (4,000 mg/kg) given as a 50% solution in mineral oil by gavage. The animals exhibited piloerection and lassitude three to four hours after treatment. They were sacrificed 1, 4, 8, 12 or 24 hours following treatment. Necropsies were performed on all animals. Microscopic examination of the lungs of treated rats revealed perivascular edema and mononuclear infiltration in the first four hours after treatment. These areas were local, but were estimated to involve 10% of the parenchyma. Areas of atelectasis and intraalveolar hemorrhage involving 15% to 20% of the parenchyma were observed 8 to 12 hours after treatment.

Electron micrographs of rat lungs after CCl_4 ingestion showed granular pneumocytes containing swollen inclusions with decreased osmiophilia and attenuated lamellae one hour after treatment. These changes were more severe four hours following treatment. By four to eight hours after treatment, cytoplasmic edema, dislocation of dense ribosomal aggregates and mitochondrial disruption were apparent. Multivesicular bodies were "conspicuously decreased" within the granular pneumocytes. Necrosis was evident 12 to 24 hours after treatment. One hour after administration, endothelial cells displayed markedly increased pinocytotic vesicles. Severe disruption of endothelial cells was evident from eight hours onward. Ultrastructural damage was seen in all components of the alveolar wall, and fibrin was observed within alveoli. The authors interpreted these findings as indicative of significant alterations in vascular permeability.

The pulmonary toxicity of orally administered CCl_4 was reported by Chen et al. (1977). Male Sprague-Dawley strain rats, weighing approximately 250 g, were given CCl_4 as a 50% solution in mineral oil at a dose of 0.25 mL CCl_4/100 g (4,000 mg CCl_4/kg) by gastric intubation. Morphologic and biochemical studies were performed at several intervals from four hours to seven days after treatment. The content of pulmonary microsomal cytochrome P-450 was decreased to 18% of control by four hours, the earliest point examined. This value fell to 8.5% of control at 12 hours and remained low for 48 hours before recovering to 75.5% of control at seven days (168 hours). Pulmonary microsomal N-demethylation activity followed a similar course. Only a transient drop in microsomal b_5 content was observed. During the seven days after gavage, there was "no striking disturbance of the (pulmonary) cellular architecture." However, morphologic analysis revealed focal areas of alveolar collapse, septal edema and transudation with no indications of cell death. Atypical type II pneumonocyte configurations were similar to previously reported changes (Gould and Smuckler 1971). These structural changes had resolved by seven days following exposure.

B. Chronic Toxicity

Alumot et al. (1976) fed groups of 36 rats (18 male and 18 female littermates) mash containing CCl_4 at 0, 80 or 200 ppm (mg/kg) of feed. The feed was stored in airtight containers, assayed for CCl_4 content and consumed soon after removal to feeding troughs. Based on measurements of food consumption and body weight, and accounting for CCl_4 loss from food prior to consumption,

the authors calculated that feed containing 200 ppm resulted in a daily dose of 10 to 18 mg/kg body weight. After two years, the surviving animals were sacrificed. In these animals, serum values for glucose, protein, albumin, urea, uric acid, cholesterol, SGOT and SGPT in the exposed animals did not differ from those in controls. No histological examination was performed. Measurement of total liver fat content did not reveal any increase in the treated animals. Thus, the authors found no biochemical abnormalities attributable to CCl_4 exposure. However, interpretation of the results was complicated by the widespread incidence of chronic respiratory disease in the animals. More than half the animals were dead at 21 months, although at 18 months, the survival ranged from 61% to 89%. The authors indicated that a dose of 10 to 18 mg/kg body weight (200 ppm feed) is a no-adverse-effect level of CCl_4 over two years. However, this conclusion may be questioned because of the poor survival rate and widespread respiratory infection of experimental animals. Liver weight and triglyceride content were not evaluated in this long-term study.

Bruckner et al. (1984) investigated the chronic oral toxicity of CCl_4 in rats. Male Sprague-Dawley strain rats, initially weighing 200 to 250 g, were given CCl_4 in corn oil at doses of 0, 1, 10 or 33 mg CCl_4/kg five times weekly for 12 weeks by gastric intubation. Serum enzyme activities were measured and histopathological evaluation of the livers were performed every two weeks during the 12-week dosage period and for two weeks after cessation of treatment. A profile of serum enzyme activities is given in Table V-6. No adverse effect was noted at a dose of 1 mg CCl_4/kg. Significant increases in

Table V-6 Serum Enzyme Activities Following Oral Administration of Carbon Tetrachloride to Rats

Week of evaluation after start of study	CCl_4 dosage (mg/kg)[a]		Serum enzyme activities[b]			BUN[c]
	Daily	Total	OCT[d]	SDH[e]	GPT[f]	(mg/dL)
2	0	0	43±7	2.9±0.7	23±1	N.D.[g]
	1	10	44±13	8.3±4.0	21±1	N.D.
	10	100	31±10	7.3±1.5	25±2	N.D.
	33	330	240±56[h]	111.5±17.0[h]	117±28[h]	N.D.
4	0	0	21±6	2.6±0.1	22±1	15.6±1.5
	1	20	18±4	N.D.	23±2	17.8±1.2
	10	200	26±3	2.9±0.3	22±1	18.5±1.2
	33	660	140±29[h]	90.2±14.7[h]	75±21[h]	18.4±0.5
6	0	0	55±13	2.5±0.5	10±1	16.2±0.2
	1	30	44±5	2.4±0.0	14±2	15.4±0.8
	10	300	40±10	8.8±0.8[h]	16±2	15.4±1.3
	33	990	251±58[h]	119.3±16.9[h]	82±20[h]	15.0±0.8

continued-

[a]Rats, initial weight 200 to 250 g, were dosed orally on a daily basis, five times weekly, for a total of 12 weeks.
[b]Values are expressed as mean ± S.E. for groups of 5 to 9 rats.
[c]Blood urea nitrogen.
[d]Ornithine-carbamyl transferase activity, nmol CO_2/mL.
[e]Sorbitol dehydrogenase activity, I.U./mL.
[f]Glutamic-pyruvic transaminase activity, I.U./mL.
[g]Not determined.
[h]Significantly different from control ($P<0.05$, paired t test).

Table V-6 - continued

Week of evaluation after start of study	CCl_4 dosage (mg/kg)		Serum enzyme activities			BUN (mg/dL)
	Daily	Total	OCT	SDH	GPT	
8	0	0	18±3	2.2±0.2	21±1	N.D.
	1	40	34±9	1.6±0.3	21±1	N.D.
	10	400	30±4	4.2±1.5	19±2	N.D.
	33	1,320	151±21[a]	110.0±10.0[a]	130±41[a]	N.D.
10	0	0	28±8	3.5±0.4	18±1	14.8±0.5
	1	50	23±3	2.3±0.6	20±1	16.0±1.0
	10	500	55±10	7.6±2.5[a]	23±1	17.6±0.5
	33	1,650	148±48[a]	134.8±15.0[a]	983±451[a]	18.2±2.5
12	0	0	45±4	3.2±0.4	20±0.3	16.6±0.7
	1	60	61±12	1.9±0.1	19±1	17.1±0.6
	10	600	69±16	8.7±2.0[a]	27±2[a]	15.3±1.2
	33	1,980	247±31[a]	145.7±57.9[a]	502±135[a]	16.2±0.8
14[b]	0	0	169±33	4.7±0.5	20±2	15.1±0.5
	1	60	111±25	3.4±0.5	20±1	16.8±0.3
	10	600	169±42	4.3±0.8[a]	19±1	16.7±0.4
	33	1,980	174±24	6.0±0.6[a]	40±7[a]	14.6±2.0

[a]Significantly different from control ($P<0.05$, paired t test).
[b]Animals were not dosed during weeks 13 and 14.

Adapted from Bruckner et al. (1984).

serum sorbital dehydrogenase activity and mild hepatic centrilobular vacuolization were found with a dose of 10 mg CCl_4/kg. Severe hepatotoxicity was caused by 33 mg CCl_4/kg, with serum enzyme levels remaining elevated during the entire 12-week treatment; microscopic examination of the liver revealed extensive cirrhotic lesions characterized by bile duct proliferation, fibrosis, lobular distortion, parenchymal regeneration and hyperplastic nodules and single cell necrosis. Serum enzyme levels returned essentially to normal within two weeks after discontinuing treatment. However, hepatic fibrosis was not reversed during this recovery period.

C. Reproductive/Teratogenic Effects

No reports concerning the teratogenic action of CCl_4 following oral ingestion have been located. However, in two studies using inhalation exposure of pregnant rats at levels of 250 to 1,000 ppm CCl_4 (1.3 to 6.3 mg/L of air) for several hours per day, no teratogenic effects were observed (Gilman 1971, Schwetz et al. 1974).

Exposure to CCl_4 *in utero* has been reported to result in liver damage in rat fetuses (Bhattacharyya 1965). Subcutaneous administration of undiluted CCl_4 at 1,600 mg/kg to a pregnant rat on day 20 of gestation resulted in small areas of focal hepatic necrosis in one fetal liver 24 hours later. Similar treatment resulted in hepatic centrilobular necrosis in neonates born 48 or 72 hours after treatment of dams on day 19 or 20 of gestation.

Cambon-Gros et al. (1984) studied the effect of oral administration of CCl_4 to pregnant rats on fetal hepatic microsomal calcium sequestration. Five pregnant Sprague-Dawley rats, weighing 200 to 250 g at the date of mating, were given 2.5 mL undiluted CCl_4/kg (4,000 mg CCl_4/kg) by gastric intubation on the 20th day of gestation. After one hour, liver samples were obtained from treated and control mothers and fetuses. Calcium sequestration, cytochrome P-450 content and extent of lipid peroxidation were measured in microsomes prepared from liver tissue. Carbon tetrachloride caused a 64% inhibition of microsomal calcium uptake in the mother, whereas fetal calcium uptake was reduced 21%. Likewise, the content of maternal cytochrome P-450 was decreased 29% by CCl_4 exposure. Fetal cytochrome P-450 levels, normally low in this tissue, were reduced to 40% of control. Carbon tetrachloride induced lipid peroxidation in maternal hepatic lipids, but not in fetal tissue. No histopathological evaluation of maternal or fetal tissue was reported in this study.

Exposure of newborn and neonatal rats to CCl_4 was studied by Dawkins (1963). Newborn (within 12 hours of birth) or 7-day-old rats (delivered from Sprague-Dawley strain mothers) were given 0.2 mL undiluted CCl_4/100 g (3,200 mg CCl_4/kg) by subcutaneous injection. Liver damage (pooled samples from at least four rats) was assessed by measuring total hepatic fat content, glucose-6-phosphatase activity and serum isocitric dehydrogenase levels and by histological evaluation. Six hours after treatment, total liver fat in the newborn was unchanged, whereas in 7-day-old rats, a small increase was noted. Serum isocitric dehydrogenase activities 24 hours after CCl_4 administration

were raised 1.5-fold in newborn rats, whereas 7-day-old rats exhibited a 15-fold increase compared to control neonates. Carbon tetrachloride did not affect glucose-6-phosphatase levels in 6-hour-old or 24-hour-old neonates. Histological findings correlated with the chemical studies.

The hepatotoxicity of CCl_4 in developing rats was reported by Cagen and Klaassen (1979). Male Sprague-Dawley rats of 1, 4, 10, 14 and 21 days of age and adult rats were given CCl_4 in corn oil by intraperitoneal injection at a dose of 1 mL CCl_4/kg (1,600 mg CCl_4/kg). Twenty-four hours after treatment, plasma enzyme activities and liver triglyceride content were measured and hepatic histopathology was assessed. Plasma aspartate aminotransferase activity and liver triglycerides were significantly increased after CCl_4 administration in each age group except the 1-day-old neonates. Likewise, histopathological examination of poisoned animals revealed "extensive pathological changes" at all ages except the 1-day-old group. Thus, the 1-day-old rat appears to be resistant to certain hepatotoxic actions of CCl_4.

Carbon tetrachloride can apparently be transferred to the neonates through mother's milk (Bhattacharyya 1965). Subcutaneous administration of CCl_4 at 1,600 or 3,200 mg/kg to four nursing rats resulted in hepatic damage in the neonates 24 or 48 hours later. A dose of 800 mg/kg to dams did not produce any hepatic damage to offspring.

Chatterjee (1966) observed testicular degeneration in rats receiving CCl_4 at 0.15 mL CCl_4/100 g (2,400 mg/kg) intraperitoneally (frequency unstated).

One group of six male rats received CCl_4 as a 1:1 mixture in coconut oil; a control group received only an equal volume of coconut oil. On day 15, all animals were sacrificed. Body weights were similar for treated and control animals. However, the relative testes weight decreased from 15.5 ± 0.4 g/kg in controls to 9.8 ± 1.2 g/kg in exposed animals. Relative weight of seminal vesicles showed an even more pronounced decrease from 1.27 ± 0.059 g/kg in control animals to 3.10 ± 0.17 g/kg in treated animals. Relative pituitary weight was increased to 50 ± 1.4 mg/kg in treated animals, as compared to 32.4 ± 0.9 mg/kg in control animals. Histological examination of testes showed testicular atrophy and "some abnormality" in spermatogenesis in CCl_4-treated animals. The authors proposed a mechanism for CCl_4-induced testicular atrophy in which blockage of pituitary hormone release results in atrophy of Leydig cells, followed by an abnormal spermatogenesis.

Kalla and Bansal (1975) gave intraperitoneal doses of CCl_4 to male rats (2,400 mg/kg as a 1:1 mixture in coconut oil) for 10, 15 or 20 days (Group I, II or III). The numbers of rats in each group were not reported. Frequency of dosing was not stated, but is assumed to be one dose/day. Controls were administered equal volumes of coconut oil. Weights of testes, seminal vesicles, epididymis and prostates were decreased in exposed animals, whereas the weight of adrenal glands increased (Table V-7). Quantitative histological studies of testes revealed that the total germinal area was higher in control animals than in treated animals and that the ratio of germinal to nongerminal area in treated rats decreased from Group I to Group III. Histological examination did not reveal any abnormalities in testes from Group I. Clusters

Table V-7 Weight Changes in Male Reproductive Organs of Rats After Carbon Tetrachloride Treatment[a]

Treatment[b] and period	Body weight (g) Before treatment	Body weight (g) After treatment	Testis (g/kg)	Seminal vesicles (g/kg)	Epididymis (g/kg)	Prostate (g/kg)	Adrenal (g/kg)	GSI[c]
Group I								
10 days								
Control	257±5	269±4.5	12.36±0.94	4.25±0.55	4.45±0.45	2.1±0.7	0.18±0.02	7.94±0.45
Treated	257±13.71	247±10.76	8.84±1.04	2.41±0.14	2.88±0.38	1.26±0.24	0.24±0.03	5.68±0.31
Group II								
15 days								
Control	230±15.49	235±5.0	10.47±0.59	3.9±0.52	4.12±0.18	2.19±0.31	0.22±0.01	6.02±0.21
Treated	230±15.49	173±13.42	9.46±0.33	1.17±0.26	2.63±0.18	1.68±0.13	0.32±0.05	5.44±0.28
Group III								
20 days								
Control	234±5.5	235±6.5	11.37±0.06	3.24±0.31	3.72±0.09	1.78±0.04	0.12±0.01	6.64±0.23
Treated	230±3.5	210±0.5	9.9±0.65	1.53±0.28	2.52±0.22	1.29±0.01	0.25±0.02	5.69±0.38

[a]Values given as mean ± S.D.
[b]Daily intraperitoneal administration of 2,400 mg CCl_4/kg as a 1:1 mixture in cocohut oil. Controls received an equal volume of coconut oil.
[c]Gonadosomatic index (body weight x weight of testes)/100.

Adapted from Kalla and Bansal (1975).

of mature sperm were present in the lumen. In Group II, slight testicular damage, a decrease in spermatogenic cells and increased lumen size were observed. In Group III, shrinkage of the tubules and increased area of the lumen were observed. Arrangement of the germ cells was disrupted; early gonadal cells were present in the lumen of many of the tubules. No spermatids were observed. Interstitial material was "damaged" and in many places the basement membrane was detached from the epithelium. Thus, a dose of 2,400 mg/kg/day for ten days had a distinct but minor effect on male rat reproductive physiology, whereas after 20 days, there were severe disturbances of spermatogenesis.

No reproductive effects were noted in rats fed diets containing CCl_4 at either 80 or 200 mg/kg of feed for up to two years (Alumot et al. 1976). Groups of 18 female rats were mated at three months of age (after six weeks of exposure) with untreated males. Thereafter, they were mated every two months with groups of treated males. Male fertility as well as reproductive activity of both sexes were generally unaffected by treatment.

D. Mutagenicity

Results of studies to determine the mutagenic activity of CCl_4 in the Salmonella typhimurium system have been uniformly negative (see reviews by McCann et al. 1975, Fishbein 1976, Rinkus and Legator 1979, Barber et al. 1981). Carbon tetrachloride has also been reported to be void of mutagenic activity when tested in Escherichia coli (Uehleke et al. 1977).

Uehleke et al. (1977) studied the interaction of CCl_4 in a liver microsome system used in conjunction with assays in S. typhimurium strains TA 1535 (test for base pair substitutions) and TA 1538 (tests for frame shift mutations). The authors incubated ^{14}C-labeled CCl_4 (1 mM) with liver microsomes (5 mg protein/mL) from rabbits, pretreated with phenobarbital, and a NADPH-regenerating system at 37°C for 60 minutes. A total of 10% of the radioactivity from CCl_4 was irreversibly (covalently) bound to microsomal protein, and more than 30% was bound to microsomal lipid. No mutagenic activity was observed in S. typhimurium strains TA 1535 or TA 1538 which were incubated with 8 mM CCl_4 and microsomal suspensions. The authors concluded that a reactive species generated in the biological system may not distribute into the incubation medium, and thus may be inaccessible to the test bacteria. They also speculated that the active products of CCl_4 may have very short half-lives.

Callen et al. (1980) reported that CCl_4 induced a mutagenic response in Saccharomyces cerevisiae strain D7. Unlike the Salmonella system, in which promutagens must be treated with exogenous liver S-9 activation systems, the D7 strain of S. cerevisiae contains an endogenous cytochrome P-450 dependent mono-oxygenase activation system. In addition, strain D7 can be used to detect both gene crossover and mitotic recombination. Three concentrations of CCl_4 (21, 28 and 34 mM) were incubated at 27°C for either one or four hours in aliquots of the yeast cell suspension (3×10^7 cells/mL). Treatment was terminated by the addition of ice-cold buffer, followed by centrifugation to remove the cells from the media. The resuspended cells were then plated on

appropriate media to allow for estimation of mutagenic activity. Based on analysis of cytochrome P-450 spectra of cell suspensions, the authors concluded that CCl_4 was absorbed and metabolized by the cells. The one-hour treatment of cells with CCl_4 resulted in significant increases in gene crossover and mitotic recombination. Results are presented in Table V-8. When cells were treated with increasing CCl_4 concentrations, decreased cell survival and increased incidence of gene conversion and mitotic recombination were observed. There was a nonlinear correlation between cell survival and the concentration of CCl_4 in the incubation mixture. It should be pointed out that, due to poor survival of cells exposed to 34 mM CCl_4, the values for gene conversion and mitotic recombinants may be artificially high. Only marginal mutagenic activity was noted when incubation was continued for four hours (data not reported by authors). This observation could have resulted from toxicity masking the genetic effects, the continued metabolism of active metabolites to inactive products or the destruction of the metabolic system. Callen et al. (1980) noted that CCl_4 had been reported to be inactive in test systems which used an exogenous mammalian activation system and suggested that their yeast system was more sensitive than some of the other *in vitro* test systems.

Carbon tetrachloride did not induce chromosome damage (i.e., chromatid gaps, deletions or exchanges) in an *in vitro* chromosomal assay using cultured rat-liver cells (Dean and Hodson-Walker 1979). Mirsalis and Butterworth (1980) found that oral exposure of adult male Fischer-344 rats (200 to 250 g) to CCl_4 (10 or 100 mg/kg) produced no increase in unscheduled DNA synthesis in

Table V-8 Mutagenic Effects of Carbon Tetrachloride on Strain D7 of *Saccharomyces cerevisiae*[a]

	CCl_4 concentration (mg/L)			
	0	3,300	4,500	5,400
Survival				
Total colonies	1,454	1,252	1,120	152
% of control	100	86	77	10
trp 5 locus (gene conversion)				
Total convertants	285	331	350	506
Convertants/10^5 survivors	2.0	2.6	3.1	61.7
ade 2 locus (mitotic recombination)				
Total twin spots	1	3	3	10
Mitotic recombinants/10^4 survivors	1.6	5.3	5.8	40.1
Total genetically altered colonies	11	19	16	65
Total genetically altered colonies/10^3 survivors	1.7	3.4	3.1	33.3
ilv 1 locus (gene reversion)				
Total revertants	38	41	57	11
Revertants/10^6 survivors	2.6	3.3	5.1	7.2

[a]The total number of colonies in the different classes represent total counts of colonies from five plates in the case of survival, conversion and revertant-frequency estimations. Mitotic recombination was estimated from counts of colonies growing on a total of 30 plates, 20 plates containing medium of which all surviving cells grew and 10 plates containing medium on which only trp 5 convertants grew. Initial cell suspensions were incubated with carbon tetrachloride for one hour at 37°C.

Adapted from Callen et al. (1980).

hepatocytes isolated from the treated rats and grown in culture. According to the authors, these results indicate that CCl_4 does not act through a genotoxic mechanism and confirm the report of Craddock and Henderson (1978) that this chemical does not induce DNA repair in hepatocytes immediately following treatment.

E. Carcinogenicity

The carcinogenic effects of CCl_4 in rats were reported by NCI (1976). Groups of 50 Osborne-Mendel rats of each sex were administered CCl_4 in corn oil by gavage five times weekly for 78 weeks at two dose levels: 47 and 94 mg/kg for males, 80 and 160 mg/kg for females. This treatment resulted in marked toxicity: at 110 weeks at the highest dose, only 7 of 50 males and 14 of 50 females survived as compared to 26 of 100 males and 51 of 100 females for controls. The incidence of hepatocellular carcinomas was increased in animals exposed to CCl_4 as compared to pooled, colony controls (Table V-9). Absolute incidence of hepatic neoplasms was low. The apparent decrease in the incidence of hepatocellular carcinomas in female rats at the high dose was attributed to increased lethality (i.e., females died before tumors could be expressed). The incidence of other neoplasms in these rats was acknowledged, but not quantified.

The effect of CCl_4 on tumors in mice was also investigated (NCI 1976). Groups of 50 B6C3F1 mice of each sex were administered CCl_4 in corn oil by gavage five times weekly for 78 weeks at two doses: 1,250 or 2,500 mg/kg

Table V-9 Incidence of Liver Tumors in Carbon Tetrachloride-Treated Rats and Colony Controls

Animal group	CCl_4 dosage[a]	Hepatocellular carcinoma	Neoplastic nodule
Males	Control (vehicle)	1/99	0/99
	Low dose (47 mg/kg)	2/50	2/50
	High dose (94 mg/kg)	2/50	1/50
Females	Control (vehicle)	0/98	2/98
	Low dose (80 mg/kg)	4/49	2/49
	High dose (160 mg/kg)	1/49	3/49

[a]Single, daily dose administered by gavage five times per week for 78 weeks. Control rats received corn oil in the same manner.

Adapted from NCI (1976).

(both males and females). High exposure-related lethality occurred in all groups. At 78 weeks at the high dose, only 2 of 50 males and 4 of 50 females survived, as compared to 53 of 77 males and 71 of 80 females for controls. After 91 to 92 weeks at the high dose, none of 50 males and 1 of 50 females survived, as compared to 38 of 77 male and 65 of 80 female controls. An exposure-related increase in the incidence of hepatocellular carcinomas was observed (Table V-10). The average latency for appearance of liver tumors was also significantly decreased in CCl_4-exposed animals. In high-dose males, the latency was 26 weeks, as compared to 48 weeks in low-dose males and 72 weeks in control males. In high-dose females, the latency was 19 weeks, as compared to 16 weeks in low-dose and 90 weeks in control female mice.

The carcinogenicity of CCl_4 in hamsters was investigated by Della Porta et al. (1961). Groups of ten Syrian golden hamsters of each sex were administered 20 mg of CCl_4 (5% solution in corn oil) by gavage weekly for seven weeks, followed by 10 mg/week for 23 weeks (equivalent to 200 and 100 mg/kg/week). Survivors were sacrificed at 55 weeks. Postnecrotic cirrhosis was observed in all animals that died in week 41. Postnecrotic cirrhosis was described by the authors to involve "regenerative hyperplastic nodules." Histological examination revealed that these nodules were characterized by obliteration of normal lobular architecture and were surrounded by fibrous tissues; cells were irregular in shape and size; nuclei and cytoplasm stained abnormally with uneven distribution of glycogen. Each of the other ten animals had one or more hepatic carcinomas. A total of 22 neoplasms was observed: 12 in five females and 10 in five males. Some were sizeable,

Table V-10 Comparison of Hepatocellular Carcinoma Incidence in Carbon Tetrachloride-Treated Mice and Colony Controls

Animal group	CCl_4 dosage[a]	Hepatocellular carcinoma
Males	Control (vehicle)	5/77
	Low dose (1,250 mg/kg)	49/49
	High dose (2,500 mg/kg)	47/48
Females	Control (vehicle)	1/80
	Low dose (1,250 mg/kg)	40/40
	High dose (2,500 mg/kg)	43/45

[a]Single, daily dose administered by gavage five times per week for 78 weeks. Control mice received corn oil in the same manner.

Adapted from NCI (1976).

measuring 5 to 30 mm. Thus, Syrian golden hamsters appear sensitive to the carcinogenic effects of CCl_4. Although the number of animals in this study was small, the authors considered the results to be significant because the reported historical control rate of hepatic tumors in hamsters was 0/254.

Andervont (1958) reported that CCl_4 was carcinogenic in C3H mice. Groups of 30 to 77 female or male C3H mice were administered 6.46 mg of CCl_4 (as a 2% solution in olive oil) by gavage once weekly for two weeks, followed by administration of 9.6 mg (as a 3% solution in olive oil) once weekly for 17 weeks (equivalent to 213 and 320 mg/kg/week). Pathogen-free or normal C3H mice were used. In males, no difference in the incidence of hepatomas was observed between pathogen-free and normal mice: 79% as compared to 49% in controls. The average number of hepatomas per tumor-bearing animal was 1.8 in treated animals and 1.3 in controls. In females, a difference between the incidence of hepatomas in pathogen-free and normal rats was observed: 46% and 29%, respectively, as compared to 3% in controls. The average number of hepatomas per tumor-bearing mouse was 1.5, 1.2 and 1.0, respectively, indicating that both the incidence and the average number of tumors per animal increased in the order: controls < treated normal mice < treated pathogen-free mice.

Eschenbrenner and Miller (1946) noted liver necrosis and hepatomas after NCI strain A mice were treated with CCl_4 for four months. Groups of five male or female mice were administered CCl_4 (in olive oil) by gavage, either 120 doses of 0, 10, 20, 40 or 80 mg/kg in 119 days (120/119) or 30 doses of 0,

40, 80 or 160 mg/kg in 116 days (30/116). Animals were sacrificed 30 days after cessation of administration. The incidence of hepatomas and necrotic lesions is shown in Table V-11. At the three highest doses in the 120/119 dose schedule, a 100% incidence of hepatomas was observed. At the highest dose, the mice had numerous hepatomas of up to 1 cm in diameter. At the two intermediate doses, the neoplasms were less frequent and smaller in size. No hepatomas were observed upon gross examination in the 10-mg/kg group. The incidence of hepatomas was less in the animals on the 30/116 schedule as compared to those on the 120/119 schedule. At the 40-mg/kg dose, however, very small hepatomas were detected by microscopic examination in two males. The authors concluded that repeated liver necrosis and its associated chronic regenerative state are probably not necessary for the induction of tumors with CCl_4.

Edwards (1941) reported the induction of hepatomas in mice by exposure to CCl_4. Of 143 female C3H mice administered 64 mg of CCl_4 (as a 40% solution in olive oil) two or three times weekly for 36 to 55 doses, 126 (88.1%) developed hepatomas. In a similar experiment, a 100% incidence of hepatomas was observed in 54 male and female strain A mice having received 23 to 58 doses. These results are summarized in Table V-12. The first hepatoma was observed following 23 doses. Tumors were usually multiple (with as many as eight in one liver) and varied in diameter from 2 to 20 mm. Peritoneal implants or metastases were not found. Under microscopy, some tumor cells were small and vacuolated and contained fat and glycogen, while others were larger, acidophilic and non-vacuolated. Few mitoses were seen, and no tumor giant cells

Table V-11 Susceptibility of Strain A Mice to Liver Necrosis and the Incidence of Hepatomas Following Oral Administration of Carbon Tetrachloride[a]

Sex	Dosage conditions[b] (doses/days)	CCl_4 dosage (mg/kg) Daily	Total	Probable initial liver necrosis[c]	Observed final liver necrosis	Hepatoma incidence
Female	120/119	0	0	-	0/5	0/5
		10	1,200	-	0/5	0/5
		20	2,400	±	0/5	5/5
		40	4,800	+	0/5	5/5
		80	9,600	+	0/5	5/5
Male	120/119	0	0	-	0/5	0/5
		10	1,200	-	0/5	0/5
		20	2,400	±	0/5	5/5
		40	4,800	+	0/5	5/5
		80	9,600	+	0/4	4/4
Female	30/116	0	0	-	0/5	0/5
		40	1,200	+	0/5	0/5
		80	2,400	+	2/5	3/5
		160	4,800	+	2/4	2/4
Male	30/116	0	0	-	0/5	0/5
		40	1,200	+	0/5	0/5[d]
		80	2,400	+	3/5	4/5
		160	4,800	+	4/4	3/4

[a]Liver samples obtained 30 days after cessation of treatment. Results based on gross and microscopic examination.
[b]Animals received 120 daily doses in 119 days or 30 doses in 116 days at intervals of 4 days.
[c]Relative probability that liver necrosis had already occurred 24 hours after one or two daily doses, based on a separate companion study.
[d]Two animals showing no tumor on gross examination were each found to have a very small hepatoma on microscopic examination of a random section of the livers.

Adapted from Eschenbrenner and Miller (1946).

Table V-12 Incidence of Hepatomas in Mice Following Oral Administration of Carbon Tetrachloride

Strain	CCl_4 dosage[a]			Age at autopsy[b] (months)	Incidence of hepatoma
	No. of doses	Each dose	Total dose		
C3H	36-55	64 mg	2,300-3,520 mg	6.0-10	126/143 (88%)
	39-50	Control	-	9-11	1/23 (4%)
A	23-58	64 mg	1,470-3,710 mg	4.5-12	54/54 (100%)
	38	Control	-	5.5	0/22 (0%)

[a] Mice were given 0.1 mL of a 40% olive oil solution containing 64 mg CCl_4 by stomach tube two or three times weekly over a period of 8 to 16 weeks. Control mice received an equal volume of olive oil.
[b] Animals killed and autopsied 2 to 147 days after last treatment.

Adapted from Edwards (1941).

were present. The tumor pattern resembled epithelial cords. Hepatomas were sharply circumscribed, but not encapsulated. No invasion of blood vessels was encountered.

Induction of hepatomas in strain L mice following oral administration of CCl_4 was reported by Edwards et al. (1942). Seventy-three male and female mice were given 64 mg CCl_4 (as a 40% solution in olive oil) by stomach tube two or three times weekly for a total of 46 doses (2,950 mg CCl_4) over a period of four months. Incidence of hepatomas was determined in two groups, 2.5 to 3.5 months and 3.5 to 7.5 months of age at the start of treatment. Results are given in Table V-13. In the younger group, 10 of 23 mice developed hepatomas, with males showing a slightly higher incidence (46.7%) than females (37.5%). In the older group, 24 of 50 mice developed hepatomas, with males showing a decidedly greater incidence (53.8%) as compared to females (27.3%). The incidence of spontaneous hepatomas in this strain was very low. Of 152 untreated male and female strain L mice autopsied, only two animals developed hepatomas (1.3%). The gross and microscopic appearance of these tumors was similar to the CCl_4-induced hepatomas seen in C3H and A strain mice reported earlier by Edwards (1941).

Reuber and Glover (1970) studied the carcinogenicity of CCl_4 in five strains of rat: Japanese, Osborne-Mendel, Wistar, Black and Sprague-Dawley. Groups of 12 to 17 male rats of each strain were given twice weekly subcutaneous injections of CCl_4 (2,100 mg/kg) as a 50% solution in corn oil. Treated animals were sacrificed when moribund; controls for each strain were sacrificed at the time as the last experimental animal. Lesions of the liver

Table V-13 Incidence of Hepatomas in Strain L Mice Following Oral Administration of Carbon Tetrachloride[a]

Sex	Age at start of experiment[b] (months)	Incidence of hepatoma[c]
Male	2.5-3.5	7/15 (46.7%)
Female	2.5-3.5	3/8 (37.5%)
Male	3.5-7.5	21/39 (53.8%)
Female	3.5-7.5	3/11 (27.3%)

[a]Mice were given 0.1 mL of a 40% olive oil solution containing 64 mg CCl_4 by stomach tube two or three times weekly for a total of 46 doses (2,950 mg CCl_4) over a period of four months.

[b]Animals killed and autopsied 3 to 3.5 months after last treatment.

[c]Incidence of spontaneous hepatomas in 152 virgin and breeding male and female strain L mice from 12 to 23 months of age was 1.3%.

Adapted from Edwards et al. (1942).

or other organs were not observed in control animals. The observed incidence of hepatic lesions in treated animals is given in Table V-14. The data suggest that sensitivity to CCl_4-induced hepatic neoplasms varies widely among strains, but this may be due in part to varying survival times among strains. All experimental animals of the Black rat strain were dead by 18 weeks, and those of the Sprague-Dawley strain by 16 weeks. The failure to find carcinomas in those strains may have been caused by an insufficient latency time. In the three other strains, lethality decreased in the same order that carcinogenicity increased.

Extrahepatic neoplasms were observed in Osborne-Mendel and Japanese strain rats. Hemangiomas of the spleen were seen in two Japanese strain rats and one Osborne-Mendel strain rat. Carcinomas of the thyroid gland were observed in three Japanese strain rats and three Osborne-Mendel strain rats. Multicystic kidneys were observed in two Osborn-Mendel strain rats. Multicystic kidneys were observed in two Osborne-Mendel strain rats and three Japanese strain rats. One Japanese strain rat had a subcutaneous leiomyosarcoma.

F. Summary

Carbon tetrachloride is toxic to animals, with oral lethality values ranging from an LDL_0 of 1,000 mg/kg in dogs to an LD_{50} of 12,800 mg/kg in mice. The tissue most affected by CCl_4 is the liver. Using release of liver enzymes into serum and histological examination as endpoints, single oral doses of 40 mg CCl_4/kg did not produce adverse effects, while doses of

Table V-14 Incidence of Hepatic Carcinoma and Cirrhosis in Five Strains of Rat Following Subcutaneous Administration of Carbon Tetrachloride[a]

EFfect of CCl_4 exposure	Strain				
	Japanese	Osborne-Mendel	Wistar	Black	Sprague-Dawley
No hyperplasia	0/15	0/13	0/12	4/17	8/16
Hyperplasia	0/15	1/13	1/12	6/17	6/16
Hyperplastic nodule	3/15	4/13	7/12	7/17	2/16
Small carcinoma	4/15	4/13	3/12	0/17	0/16
Large carcinoma	8/15	4/13	1/12	0/17	0/16
Total carcinoma	12/15	8/13	4/12	0/17	0/16
No cirrhosis	0/15	0/13	0/12	0/17	0/16
Mild cirrhosis	9/15	2/13	0/12	0/17	0/16
Moderate cirrhosis	5/15	7/13	6/12	4/17	0/16
Severe cirrhosis	1/15	4/13	6/12	13/17	16/16

[a]Animals were dosed twice weekly with 2,080 mg CCl_4/kg, given by subcutaneous injection in corn oil.

Adapted from Reuber and Glover (1970).

80 mg/kg or higher did. Numerous studies have reported that oral doses ranging from about 100 to 4,000 mg/kg produce fatty infiltration, loss of cytochrome P-450 and other enzymes, inhibition of protein synthesis and histological alterations in the liver. High doses were often found to produce irreversible damage and hepatocellular necrosis, but the effects observed following lower doses were largely reversible.

Kidney and lung are also affected following oral exposure to CCl_4. Doses of about 4,000 mg/kg resulted in lesions of the renal proximal tubule and pulmonary Clara cells and endothelial cells. These changes also appeared to be reversible when damage was not too severe.

The effects of chronic exposure to CCl_4 are similar to the effects of acute exposure: the liver is the most sensitive tissue, showing fatty infiltration, release of liver enzymes, inhibition of cellular enzyme activities, inflammation and ultimately cell necrosis. Rats exposed to doses of 1 mg CCl_4/kg for 12 weeks did not show measurable adverse effects, while doses of 10 or 33 mg/kg produced enzyme release, centrilobular vocuolization and necrosis.

Carbon tetrachloride has not been shown to be teratogenic. Newborn animals appeared to be less sensitive to CCl_4 than older animals. An intraperitoneal dose of 2,400 mg/kg had adverse effects on testicular function.

No evidence of mutagenic activity for CCl_4 has been found in bacterial test systems or in cultured liver cells, although increased gene crossover and mitotic recombination were observed in yeast cells exposed to 3,200 to 5,200 mg CCl_4/L.

Carbon tetrachloride is carcinogenic in animals, producing mainly hepatic neoplasms. Doses of about 30 mg/kg/day or higher for six months or longer have been found to produce increased frequency of hepatomas, hepatocellular adenomas and hepatocellular carcinomas in mice, rats and hamsters.

VI. HEALTH EFFECTS IN HUMANS

A. Clinical Case Studies

1. Ingestion

Lamson and Minot (1928) studied the lethal effects of CCl_4 in patients receiving CCl_4 and magnesium sulfate orally as a treatment for hookworms. The authors reported the treatment of thousands of patients with a single dose of 2.5 to 15 mL (4.0 to 24 g) of CCl_4 to be without ill effects. One man was reported to have safely ingested 40 mL (64 g) of CCl_4. However, an "extremely small" population of adults died after receiving 1.5 mL (2.4 g) of CCl_4, and doses of 0.18 to 0.92 mL (0.29 to 1.5 g) were reported to be fatal to children. Susceptibility in adults was correlated with alcohol intake (chronic alcoholism or exposure to alcohol shortly after treatment), the presence of ascarid worms and the intake of foods, particularly those with fat content.

A fatality attributed to ingestion of CCl_4 was reported by Smetana (1939). The victim, a photographer described as having "a history of chronic alcoholism," died ten days after consuming an unknown amount of "some fluid containing carbon tetrachloride." He presented symptoms including nausea, vomiting, jaundice, anuria and semistupor. In the final clinical diagnosis, death was attributed to CCl_4 poisoning, with acute hepatic yellow atrophy, nephrosis and pulmonary edema.

A case of attempted suicide by ingestion of CCl_4 was reported by Stewart et al. (1963). The victim, a 29-year-old female who ingested one pint of a CCl_4:methanol solution (2:1), experienced ringing in the ears immediately after ingestion and lost consciousness. She was hospitalized for three weeks. Three hours after ingestion, CCl_4 in the exhaled breath and blood was confirmed by infrared analysis. Carbon tetrachloride in the exhaled breath was then monitored throughout the hospitalization, and was reported to decrease exponentially. Because of the toxicity of the methanol and the possibility of synergistic reactions with the CCl_4, hemodialysis was performed soon after admission. Mannitol solution was given by continuous intravenous infusion. Clinical laboratory analyses during hospitalization showed some elevation of SGOT, which reached a maximum of 75 units at day 6, and an elevation of urinary urobilinogen to a maximum of 7.8 Ehrlich units at day 10. Other laboratory findings included elevation of serum iron and depression of serum protein concentration and albumin fractions. The retention time of bromosulfophthalein was increased. These findings were interpreted as evidence of minimal hepatocellular injury. Acute renal dysfunction was not observed; the authors credited the mannitol treatment with preventing renal damage.

The effects of oral administration of CCl_4 on the liver and kidneys of humans have been evaluated in condemned male prisoners in Ceylon (Docherty and Burgess 1922, Docherty and Nicholls 1923). Three prisoners were given 4 mL CCl_4 and two were given 5 mL CCl_4. Assuming a body weight of 70 kg, these doses correspond to 91 or 114 mg/kg. One prisoner was given 5 mL plus an additional 3 mL two weeks after the initial dose. The prisoners were

executed 3 to 15 days after CCl_4 administration, and their liver and left kidney removed at autopsy for microscopic examination. Although the kidneys of just two individuals exhibited alterations, the livers of four of the six showed fatty or granular degeneration. This study indicates that a dose of about 100 mg/kg is hepatotoxic in humans, but does not identify a NOAEL.

2. Inhalation

Human volunteers were exposed to known concentrations of CCl_4 vapor in an effort to correlate physiological and/or biochemical changes to the magnitude of exposure (Stewart et al. 1961). Eight healthy male volunteers were exposed to CCl_4 vapors in a series of three separate experiments performed one month apart. Prior to exposure, data on blood pressure, SGOT and urinary urobilinogen were obtained for each subject. Samples of preexposure exhaled breath, urine and blood showed no detectable CCl_4. The volunteers were seated in a closed room (11 x 12 x 7.5 feet) where 99% pure CCL_4 was poured into a dish and covered with a towel. An exhaust system grill and door were closed during the experiment, but an air supply grill was left open. A fan circulated air across the dish. Carbon tetrachloride ambient concentrations were monitored with a Davis halide meter and an infrared spectrometer. The CCl_4 concentration ranges and exposure times are given in Table VI-1.

Carbon tetrachloride was detected in exhaled breath in all three experiments. Graphs showed an exponential decrease in concentration of CCl_4 versus time. The exact values were not given. The concentration of serum iron

Table VI-1 Exposure Times and Concentrations of Carbon Tetrachloride Vapor in a Controlled Human Study

Experiment	Average concentration, time-weighted (mg/m^3)	Concentration range (mg/m^3)	Exposure (minutes)
1	309	192-548	70
2	69	63-88	180
3	63	57-88	180

Adapted from Stewart et al. (1961).

showed an initial decrease in three of six subjects exposed to 309 mg/m^3, but returned to normal in two of these subjects 68 hours after exposure. The remaining subject showed a 31% depression in serum iron at 68 hours, but the value was within the normal range. Serum iron was not analyzed in the other two experiments. Of the six subjects exposed to CCl_4 at 309 mg/m^3, the serum glutamic oxaloacetic transaminase (SGOT) level was slightly elevated in some and depressed in others, but remained within the normal range. Carbon tetrachloride was not detected in the blood or urine at any exposure time or dose, but the analytical technique used was not a sensitive one. The authors concluded that no ill effects were observed from exposure to CCl_4 at 63 mg/m^3 for 180 minutes, although the small changes in serum iron at the high dose (309 mg/m^3) might have been an indication of liver insult.

Bilateral peripheral constriction of the ocular color fields, resulting in symptoms of toxic amblyopia in three males, was attributed to the inhalation of CCl_4 vapors (Wirtschafter 1933). Five male employees of dry cleaning establishments who had been exposed to CCl_4 (of unknown concentration) from eight to ten hours daily for one to six months were examined. Two men also had signs of conjunctivitis. Three of the men complained of visual disturbances characterized by blurred vision or spots before the eyes. The author concluded that toxic amblyopia may result from exposure to CCl_4 vapor.

One fatality occurred in two cases of CCl_4 poisoning reported by Smetana (1939). In the fatality, a dry cleaner-interior decorator described as being "a steady and heavy drinker," was exposed for several hours to CCl_4 vapor

during work. Upon returning from work, he noted dyspnea. Several hours after the exposure, headache, dizziness and malaise developed, accompanied by nausea and repeated vomiting that persisted for several days. The patient also suffered labored breathing and cough with bloody sputum before he died 99 days following exposure.

The second inhalation case reported by Smetana (1939) was a housemaid with a history of chronic alcoholism. Three days before hospitalization, the patient cleaned dresses with CCl_4 for three hours in a poorly ventilated room. Soon after exposure, she began to vomit. She suffered symptoms similar to those described for the other victim. After approximately 1.5 months of hospitalization, this patient was released. Her condition several weeks later was described as "much improved."

Seven cases of CCl_4 poisoning reported by Norwood et al. (1950) resulted from both occupational and nonoccupational inhalation exposures. In the three cases described as "severe" poisonings, there was a history of chronic alcoholism; two fatalities occurred in this group. In one case, the victim had been exposed for about 15 minutes to an atmosphere containing CCl_4 at an estimated 1,575 mg/m^3 (this estimate was made by duplicating the conditions). Histopathologic examination of liver and kidney tissue from the fatalities revealed liver necrosis and degeneration of the renal tubules. The four remaining cases were characterized as "mild industrial" exposures. After exposure to CCl_4, all subjects suffered varied symptoms, including nausea, vomiting, diarrhea, headache, muscular ache, pain or numbness, labored breathing and dizziness.

In another case, a 31-year-old janitor suffered malaise, back and lower abdominal pain, nausea and vomiting the morning after working with CCl_4 for five hours in a closed room (Kittleson and Borden 1956). He reportedly consumed two bottles of beer during the exposure period. The patient required two months of hospitalization for treatment of acute renal insufficiency as a result of the CCl_4 poisoning.

Elevated SGOT activities with concomitant liver changes were reported in two men occupationally exposed to unreported concentrations of CCl_4 (Lachnit and Pietschmann 1960). One became ill after exposure to CCl_4 for three hours in a relatively well-ventilated room. He was hospitalized three days after exposure. His liver was slightly enlarged, and his SGOT value was elevated by 6,000 units. This value rapidly decreased and returned to normal by the tenth day. A biopsy of the liver taken on the eighth day showed necrosis in the centers of the lobuli, but the surrounding tissue was undamaged. An additional needle biopsy of the liver taken on the 28th day showed that the cells had almost returned to normal. In the second case, a male similarly exposed to CCl_4 entered the hospital 12 days after exposure. His SGOT had increased to 80 units. A liver needle biopsy on the 22nd day showed only moderate changes, some of a degenerative nature.

In a chemical packing plant, use of CCl_4 by two workers for equipment cleaning resulted in the hospitalization of 4 of 43 workers at the plant (Folland et al. 1976). Ten additional workers also became ill. Carbon tetrachloride concentrations at the time of exposure were not ascertained.

Eight of the 43 workers fell ill within 12 hours following the start of this 2-hour exposure; six others followed within the next 36 hours. The four hospitalized workers showed evidence of severe disruption of liver function: one case had an SGOT level of 13,390 units. All patients recovered within 90 days. All hospitalized workers as well as most of the others taken ill had worked near a bottle-filling operation for isopropyl alcohol at the northern end of the plant, adjacent to the CCl_4 cleaning area. The authors attributed the toxic episode to CCl_4 toxicity potentiated by isopropyl alcohol.

Tracey and Sherlock (1968) reported on a 59-year-old man with a history of moderate alcohol consumption who returned from a cocktail party and noticed the vapor of CCl_4, used to clean a rug in his apartment earlier that evening. Five days later he developed nausea, vomiting and diarrhea, and within ten days of exposure he developed jaundice. The patient recovered following a long and complex hospitalization, and was discharged after nine weeks. Four years later he was found to have a smooth, enlarged, non-tender liver. He denied alcohol consumption within the intervening period. Three years after this checkup, the patient was readmitted with a history of nausea, vomiting and diarrhea. A liver biopsy was diagnosed as hepatocellular carcinoma. No treatment was administered until readmission five months later, when he received an X-ray radiation dose of 3000 R. He died two weeks after discharge. Postmortem examination revealed the liver to be extensively involved with the tumor. Little normal liver tissue remained. The authors stated that no causal relationship between the exposure and hepatocellular carcinoma could be drawn from this case.

Other reports of human neoplasms developing after exposure to CCl_4 have appeared. In one case, a woman developed nodular cirrhosis of the liver followed by cancer of the liver after exposure to CCl_4, and died three years after the first exposure (Johnstone 1948). However, she had suffered from periodic jaundice for five years prior to exposure to CCl_4. In a second case, a fireman developed cirrhosis and an "epithelioma" of the liver four years after acute CCl_4 intoxication (Simler et al. 1964). A causal link between CCl_4 exposure and development of neoplasms could not be definitely established in any of these cases.

B. Epidemiological Studies

A retrospective study of laundry and dry cleaning workers was conducted by Blair et al. (1979) to determine if occupational exposure to CCl_4, trichloroethylene, tetrachloroethylene and petroleum solvents resulted in increased morbidity or mortality. Data for cases were obtained from union records. Sex, race, age at death and underlying and contributing cause of death were abstracted from death certificates. The age, race, sex and cause distribution for all deaths in the United States from 1957 to 1970 served as the control standard. Causes of death were analyzed using the proportionate mortality method. The results of the analysis demonstrated an excess of lung and cervical cancer and slight excesses of leukemia and liver cancer. Because of the multiple historical exposures experienced by this population, it is difficult to establish a causal association between specific substances and increased mortality trends. This study is presented as evidence of a possible

link between CCl_4 exposure and increased mortality, rather than as a study in which quantitative extrapolations of the chemical's effects on human health are possible.

A study of residents of an area surrounding a solvent recovery plant in rural Maryland revealed an increase in the incidence of lymphatic cancer (Capurro 1979). This plant emitted at least 31 chemicals (identified by gas chromatography), including CCl_4. The mortality experience of residents of a 1.5-km^2 area around the plant was followed from October, 1968, to October, 1974. Six deaths, one due to cancer, were expected over this period. Fourteen deaths were observed, including seven from cancer. Four of seven malignancies were lymphomas, more than 60 times the expected incidence. These deaths were not attributed to any particular chemical.

A cross-sectional epidemiologic study (Sonich et al. 1980) examined health effects of CCl_4 ingestion in humans. Seventy tons of CCl_4 were spilled in the Kanawha and Ohio River in 1977. Measurements of raw water revealed a maximum concentration of 0.340 mg CCl_4/L. Twenty-one cities situated along the river were involved in the study. These cities represented areas that obtained their drinking water directly from the river and/or areas that obtained their drinking water from sources not influenced by the quality of the river water. By using river volumes and flow rates, periods of high exposure (1977) and low exposure (1976) to CCl_4 were estimated for each city along the river. The results of routine tests of clinical chemistries reflecting liver and kidney function, together with basic epidemiologic information, were abstracted from

approximately 6,000 medical records. A positive and statistically significant ($P<0.05$) relationship between the CCl_4 exposure and the frequency of elevated levels of serum creatinine in exposed patients was found. Similar results were not found for any of the other parameters analyzed.

C. High Risk Subpopulations

No studies were located which provided evidence that any particular human subpopulation is at greater risk than the general population, except that alcoholics appear to be considerably more sensitive than non-alcoholics. These data have been presented above (Section VI.A.).

D. Summary

The effects of CCl_4 exposure in humans are similar to effects seen in animals, with the liver, kidney and lungs being most sensitive. Single oral doses of 2.5 to 15 mL (57 to 343 mg/kg) usually produced no external effects, although some individual adults suffered adverse effects (including death) from ingestion of as little as 1.5 mL (34 mg/kg). Doses of 0.18 to 0.92 mL (29 to 150 mg/kg) may be fatal in children.

Inhalation exposure also results in renal and hepatic damage. No ill effects resulted from three hours of exposure to 63 mg/m^3, but 70 minutes of exposure to 2,309 mg/m^3 did produce some liver effects. Higher levels (1,500 mg/m^3) produced severe poisoning and death.

No strong evidence was located indicating that CCl_4 (either oral or inhaled) produces cancer in humans, although cases of cancer in exposed individuals have been reported.

No evidence was located to indicate that any human subpopulation is more sensitive to CCl_4 than is the general population. A number of reports cited evidence that individuals who chronically consume large amounts of alcohol are considerably more sensitive than individuals who consume little alcohol.

VII. MECHANISM OF TOXICITY

The toxicity of CCl_4 to an organism depends upon the ability of the organism to metabolize the compound. Thus, unmetabolized CCl_4 does not appear to be significantly toxic (Recknagel and Glende 1973). In mammals, CCl_4 is thought to be metabolized in the endoplasmic reticulum of the liver by the mixed-function oxidase system. The proposed pathway of CCl_4 metabolism was outlined in Section III.C (Metabolism). Two free radicals have been postulated as metabolic intermediates: the trichloromethyl radical and the chlorine radical. The toxicity of CCl_4 has been attributed to subsequent reactions of the trichloromethyl radical. These reactions include formation of carbonyl chloride (phosgene), dimerizatiion to hexachloroethane, free radical binding to lipids and protein and lipid peroxidation. In this section, these proposed reactions are discussed in conjunction with the toxic effects attributed to each.

A. Formation of Carbonyl Chloride (Phosgene)

From the results of an *in vitro* study of CCl_4 metabolism, Shah et al. (1979) postulated the formation of carbonyl chloride from the trichloromethyl radical. The authors incubated L-cysteine and ^{14}C-CCl_4 with rat liver homogenate and looked for the formation of 2-oxothiozolidine-4-carboxylic acid. This compound is formed from the reaction of L-cysteine and carbonyl chloride. Analysis by mass spectroscopy of the metabolic products showed a fragmentation pattern consistent with 2-oxothiozolidine-4-carboxylic acid. The authors

inferred from these analytical data that carbonyl chloride was formed as a metabolic product of CCl_4. Although carbonyl chloride (phosgene) is not reported to be a carcinogen, the authors pointed out that the compound is highly toxic and that the reactive chlorines could react with macromolecules in ways similar to alkylating agents.

B. Dimerization to Hexachloroethane

Hexachloroethane has been identified as a metabolite of CCl_4 by Fowler (1969). The formation of this compound is believed to take place by the dimerization of the trichloromethyl radical. Although hexachloroethane is a hepatotoxin, its toxicity is less than that seen in CCl_4 poisoning. Therefore, other mechanisms probably account for the severity of the toxicity associated with CCl_4.

C. Free Radical Binding to Proteins

Free radical binding to proteins has been postulated as one cause of toxicity associated with CCl_4. As reviewed by Recknagel and Glende (1973), this binding was stated to involve reactions with cellular proteins, particularly those with sulfhydryl groups. More recently, ^{14}C- CCl_4 has been observed to bind irreversibly to rabbit microsomal proteins at a rate of approximately 20 nmol/mg protein/hour (Uehleke and Werner 1975). Binding of CCl_4 (or its metabolites) to hepatic macromolecules was enhanced in the absence of oxygen and is consistent with the proposal that the trichloromethyl radical is the reactive metabolite.

Although it has been shown that label from ^{14}C-CCl_4 binds to proteins, the question of CCl_4 binding to polynucleotides is still open. The question is important because of its implications for the mechanism of CCl_4 carcinogenicity and mutagenicity. Rocchi et al. (1973) examined the possible binding of ^{14}C-CCl_4 with nucleic acids in Wistar rats and Swiss mice *in vivo* and with DNA and polynucleotides *in vitro*. Radioactive ^{14}C-CCl_4, 367 µmol/kg (56.4 mg/kg), binds *in vivo* to DNA of mouse liver and to ribosomal RNA of rat liver if the animals have been pretreated with 3-methylcholanthrene (3-MC). The pretreatment with 3-MC increases the amount of the binding due to a higher activity of the microsomal enzyme system activating CCl_4. *In vitro*, CCl_4 (0.22 or 1.3 µmol/6 mL incubate, 5.5 or 33 µg CCl_4/mL incubate) is activated by microsomes and pH 5 enzymes of 3-MC-treated animals to a metabolite which can react DNA and polynucleotides. No binding of CCl_4 metabolites to hepatic DNA from control mice or rats was detected. On the other hand, when Uehleke and Werner (1975) incubated ^{14}C-CCl_4 with either isolated liver microsomes (rat or mouse, species not identified) or soluble RNA, they observed no ^{14}C binding to ribosomal RNA or exogenous RNA. Experimental details were not presented.

Diaz Gomez and Castro (1980) reported that ^{14}C from CCl_4 irreversibly binds *in vivo* to hepatic nuclear DNA from strain A/J mice and Sprague-Dawley rats (Table VII-1). Also, binding of ^{14}C from CCl_4 to DNA was observed *in vitro* in incubation mixtures containing microsomes and a NADPH-generating system as well as in tissue slices (Table VII-2). Liver nuclear proteins (Table VII-3) and lipids (Table VII-4) reversibly bind CCl_4 metabolites. The

Table VII-1 Covalent Binding of ^{14}C from $^{14}CCl_4$ to Rat and Mouse Liver DNA *In Vivo*[a]

Species	^{14}C from $^{14}CCl_4$ in DNA[b]	
	pmol/mg ± S.D.	mol nucleotide/mol of CCl_4 metabolite (x 10^6)
A/J mice	0.72±0.05	4.54
Sprague-Dawley rat	0.52±0.05	6.25

[a]Labeled CCl_4 (27 mCi ^{14}C/mmol) was administered intraperitoneally as an olive oil solution (25 µCi/mL) at a dose of 10 mL of solution/kg. Animals were sacrificed six hours after $^{14}CCl_4$, and DNA was isolated and counted. For calculations, it was considered that 1 mg of DNA contained 3.2 µmol of nucleotides.

[b]Results are the mean of three samples. Each sample was a pool of 10 livers in the case of mice and one liver for rats. Values for rats were significantly lower than those of mice ($P<0.05$).

Adapted from Diaz Gomez and Castro (1980).

Table VII-2 Covalent Binding of ^{14}C from $^{14}CCl_4$ to DNA *In Vitro*[a]

Experimental	^{14}C from $^{14}CCl_4$[b]	
	DNA pmol/mg ± S.D.	DNA mol nucleotide/mol of CCl_4 metabolite
Microsomal activation	1.81±0.13	1.75×10^6
Microsomal activation (33 mM CCl_4)	4,302±300	7.5×10^2
Chemical activation	826±250	3.84×10^3

[a]Anaerobic mixtures containing microsomes, NADPH and mouse liver DNA were incubated for 30 minutes at 37°C with 10 µL of an ethanol solution of $^{14}CCl_4$ (27 mCi/mmol) at a concentration of 13.63 µCi/mL. DNA was isolated and counted. In the second experiment on the microsomal activation, the incubation system was as before except that unlabeled CCl_4 was added (final concentration, 33 mM) and specific activity was 0.0137 mCi/mmol. When chemical activation was studied DNA/cetyltrimethyl ammonium bromide salts (3 mg) were heated for 16 hours at 80°C in a sealed vial under N_2 atmosphere with 3 mL of an alcoholic solution of $^{14}CCl_4$ (1.82 µCi/mL) in the presence of benzoyl peroxide. DNA was isolated and counted.

[b]All results are the mean of triplicate simultaneous experiments.

Adapted from Diaz Gomez and Castro (1980).

Table VII-3 Covalent Binding of Label from $^{14}CCl_4$ to Nuclear Protein Fractions from Rat Liver[a]

Nuclear protein fraction	^{14}C in protein[b] (pmol/mg)	Percentage total label in each fraction
I	4.9-7.5	18.25-19.95
II	17.9-10.1	29.73-23.61
III	8.8-5.3	7.76-5.33
IV	68.1-52.6	12.14-8.23
V	39.3-28.5	6.55-4.85
Histones	43.6-56.8	7.13-14.07
Residual	45.7-63.5	18.44-23.96

[a]Administration of $^{14}CCl_4$ and time of treatment as in Table VII-1. Isolated proteins were dissolved in formic acid and counted. Fractions I and II correspond to nuclear sap proteins, fractions III and IV are deoxyribonucleoproteins, fraction V is acid ribonucleoprotein.

[b]Pooled livers from four rats were used in each experiment. These results are from two separate experiments.

Adapted from Diaz Gomez and Castro (1980).

Table VII-4 Covalent Binding of ^{14}C from $^{14}CCl_4$ to Different Nuclear Lipid Fractions from Rat Liver[a]

Lipid fraction	Percentage of total label in each fraction[b]
Phospholipids	75.69-65.11
Diglycerides	16.73-24.07
Cholesterol esters	0.36-0.24
Triglycerides	0.22-0.37
Free fatty acids	1.19-0.64
Cholesterol	5.70-9.50
Undetermined or lost	0.11-0.07

[a]Administration of $^{14}CCl_4$ as in Table VII-1. Animals were sacrificed three hours after dosing. Total lipid fractions were used for counting. Covalent binding to total nuclear lipids was 113.5 pmol/mg.

[b]Pooled livers from four rats were used in each experiment. These results are from two separate experiments.

Adapted from Diaz Gomez and Castro (1980).

authors concluded that: (1) the differences between their results and those of Rocchi et al. (1973) (pretreatment with 3-MC required) and theirs were possibly related to the use of different strains of mice (A/J versus Swiss) and rats (Sprague-Dawley versus Wistar); (2) the interaction of CCl_4 metabolites with DNA and nuclear proteins could be relevant to CCl_4-induced liver tumors and hepatoxic effects; and (3) the epigenetic mechanisms for chemical induction of cancer, not involving CCl_4-DNA interactions as shown in their study, could also be relevant.

D. Lipid Peroxidation

A number of the hepatic effects resulting from CCl_4 exposure, including the fatty liver syndrome, are generally believed to arise as a result of lipid peroxidation (Recknagel and Glende 1973). The first step in the reaction sequence proposed for CCl_4-induced lipid peroxidation is the production of trichloromethyl free radical from metabolic cleavage of the carbon-chlorine bond. The trichloromethyl radical initiates a chain reaction by reacting with the hydrogen atom of a methylene group in an unsaturated fatty acid, generating a fatty acid-free radical. On reaction with molecular oxygen, the fatty acid-free radical is converted into an unstable organic peroxide. The peroxide disintegrates in two fashions: (1) intramolecular cyclization to form malonic dialdehyde and two new free radicals or (2) simple homolytic fission that also yields two free radicals. This whole process occurs autocatalytically: each free radical gives rise to two new free radicals. According to the lipid peroxidation hypothesis, it is the process of peroxida-

tive decomposition of structural lipids in the hepatic endoplasmic reticulum and other cellular membranes which is responsible for the spectrum of pathological consequences initiated by CCl_4 (Recknagel and Glende 1973).

Supporting evidence for the lipid peroxidation hypothesis has come from *in vivo* and *in vitro* experiments. Rao and Recknagel (1968) demonstrated that intragastric administration of 0.25 mL CCl_4/100 g (4,000 mg CCl_4/kg) induces marked diene conjugation absorption in lipids extracted from hepatic microsomes and mitochondria. Peroxidation of microsomal lipid was one-half maximal five minutes after treatment and complete after 15 minutes, whereas mitochondrial lipid peroxidation proceeded more slowly. An *in vitro* prooxidant action of CCl_4 has been shown by Glende and Recknagel (1969). Lipids in microsome-cytoplasmic fractions isolated from rat liver readily undergo peroxidative decomposition in the presence of CCl_4 (1.0 µL CCl_4/mL, 1.6 mg CCl_4/mL) when supplemented with a NADPH-generating system.

Experiments by Pasquali-Ronchetti et al. (1980) have also shown that the intragastric administration of CCl_4 (0.5 mL/100 g, 8,000 mg/kg) induces lipid peroxidation in rat liver endoplasmic reticulum. *In vitro* studies (Pasquali-Ronchetti et al. 1980) have demonstrated lipoperoxidative disintegration of hepatic microsomes within 15 minutes in the presence of 1 µL CCl_4/2.5 mL incubate (0.6 mg CCl_4/mL incubate).

Other indicators have been used in *in vivo* and *in vitro* assays of lipid peroxidation: pentane and ethane levels in exhaled air (arising from fatty

acid decomposition) and malonic dialdehyde concentrations in hepatocytes (arising from intramolecular cyclization). Pentane production in male rats increased by factors of 4.6, 13.2 and 26.4 over that in mineral oil controls within 30 minutes following intraperitoneal administration of CCl_4 at doses of 160, 480 and 1,440 mg/kg, respectively (Sagai and Tappel 1979).

E. Interactions

Cornish and Adefuin (1966) reported that ethanol ingestion potentiated the toxicity of subsequent exposure to CCl_4. Sixteen to 18 hours following a single dose of ethanol (5 g/kg), rats were exposed to CCl_4 vapor for two hours. Twenty-four hours later, SGOT activities were increased 2.4- to 8.5-fold over control animals (not given ethanol) at exposure levels of 100 or 1,000 ppm CCl_4, respectively. The authors stated that histopathological evaluation of the liver (degree of centrilobular necrosis and fatty infiltration of parenchymal cells) correlated closely with the serum enzyme changes 24 hours after CCl_4 exposure.

Methanol, ethanol or isopropanol treatment has been found to potentiate hepatic injury induced by CCl_4 (Traiger and Plaa 1971). A single intragastric dose of 10.0 mL methanol/kg (8,000 mg/kg), 7.0 mL ethanol/kg (5,500 mg/kg) or 2.5 mL isopropanol/kg (2,000 mg/kg) was administered 20 hours prior to an intraperitoneal injection of 0.00075 mL CCl_4/kg (1.2 mg CCl_4/kg). After 24 hours, serum glutamic-pyruvic transaminase (SGPT) activity was measured to assess liver injury. Treatment with methanol, ethanol or isopropanol produced

a 6.6-, 1.6- or 37-fold potentiation in SGPT activity in CCl_4-treated Swiss-Webster mice. Similar treatment of mice with n-propyl alcohol, n-butyl alcohol or 4-methyl-2-pentanol failed to potentiate CCl_4-induced hepatic inj[illegible] Ethanol or isopropanol treatment of rats also potentiated CCl_4-induced accumulation of hepatic triglycerides and loss of liver glucose-6-phosphatase activity, with isopropanol being the more effective potentiator.

Traiger and Bruckner (1976) reported that 2-butanol and 2-butanone potentiate the hepatotoxicity of CCl_4 in rats. Either 2-butanol (2.2 mL/kg, 1,800 mg/kg) or 2-butanone (1.87 mL/kg, 1,500 mg/kg), administered by gavage 16 hours prior to the intraperitoneal injection of 0.1 mL CCl_4/kg (160 mg CCl_4/kg), significantly enhanced CCl_4-induced liver injury. Serum glutamic-pyruvic transaminase (SGPT) activity in CCl_4-treated rats was potentiated approximately 30-fold by either 2-butanol or 2-butanone. Likewise, the CCl_4-induced loss in hepatic glucose-6-phosphatase activity and accumulation of triglyceride were potentiated by both agents. Morphologic evaluation substantiated the potentiated hepatic response to CCl_4.

Kluwe et al. (1979) reported that dietary polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) potentiate the hepatic and renal toxicity of CCl_4. Mice were fed diets supplemented with 100 ppm PBB or 200 ppm PCB for 28 days prior to a single intraperitoneal injection of CCl_4. Mice fed PCBs or PBBs were particularly responsive to doses of 0.125 mL CCl_4/kg (200 mg CCl_4/kg) and 0.625 mL CCl_4/kg (1,000 mg CCl_4/kg). Serum glutamic oxaloacetic transaminase (SGOT) activities were significantly

potentiated in both dietary groups at these CCl_4 doses. Mice consuming the PBB diet did not survive the dose of 1,000 mg CCl_4/kg. Renal dysfunction, defined as loss in capacity to accumulate p-aminohippuric acid in kidney slices, was potentiated in PBB-fed mice given 200 mg CCl_4/kg. However, CCl_4-induced renal dysfunction was not potentiated in PCB-fed animals. Dietary PBB (20 or 100 ppm) fed to mice for 20 days lowered the 96-hour LD_{50} dose of CCl_4 from 1.84 mL/kg (3,000 mg/kg) to 1.00 mL/kg (1,600 mg/kg) and 0.28 mL/kg (450 mg/kg), respectively.

Cornish et al. (1973) found that phenobarbital potentiated the hepatotoxicity of CCl_4. A single intraperitoneal injection of phenobarbital, 50 mg/kg, given to rats 24 hours prior to CCl_4 treatment enhanced liver injury as determined by SGOT activity. Several-fold potentiation of SGOT levels were observed in animals receiving 0.05 mL (80 mg) to 0.6 mL (960 mg) CCl_4/kg by intraperitoneal injection.

Chlordecone has been found to potentiate the hepatotoxicity of CCl_4 (Curtis et al. 1979). Rats were fed diets containing 10 ppm chlordecone for 15 days prior to single intraperitoneal administration of CCl_4 at doses ranging from 25 to 200 µL CCl_4/kg (40 to 320 mg CCl_4/kg). Several functional indices of liver injury were measured 24 hours later. A dose-dependent impairment of biliary excretion of phenolphthalein glucuronide was potentiated in chlordecone-fed rats given 50 µL CCl_4/kg (80 mg CCl_4/kg) or higher as compared to rats on a chlordecone-free diet. Bile flow was significantly reduced in chlordecone-fed rats given 100 and 200 µL CCl_4/kg (160 and 320 mg

CCl_4/kg). Serum glutamic-pyruvic transaminase (SGPT) and SGOT activities were potentiated in excess of 25- and 7-fold, respectively, in chlorodecone-fed rats given 160 mg CCl_4/kg.

F. Summary

The metabolism of CCl_4 is thought to proceed through the formation of the trichloromethyl free radical, but the details of the subsequent reactions that produce cell injury remain obscure. Reaction of the trichloromethyl radical with proteins and/or radical-induced lipid peroxidation are two mechanisms that may be involved. Some metabolic end products (hexachloroethylene and phosgene) are hepatotoxic, but the importance of these chemicals in CCl_4-induced cell injury is not clear.

Administration of certain compounds prior to CCl_4 exposure has been found to significantly potentiate the hepatotoxicity of CCl_4. These potentiating agents include ethanol, methanol, isopropanol, polybrominated biphenyls (PBBs), polychlorinated biphenyls (PCBs), phenobarbital and chlordecone.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of an assessment of the non-carcinogenic and carcinogenic effects. In the quantification of non-carcinogenic effects, an Acceptable Daily Intake (ADI) is calculated. An Adjusted Acceptable Daily Intake (AADI) and Health Advisory (HA) values for the chemical are then calculated to define the appropriate drinking water concentrations to limit human exposure. For ingestion data, this approach is illustrated as follows:

$$\text{ADI} = \frac{(\text{NOAEL or LOAEL in mg/kg/day})(\text{Body Weight in kg})}{\text{Uncertainty/Safety Factor}} = \text{mg/day}$$

$$\text{AADI} = \frac{\text{ADI}}{\text{Drinking Water Volume in L/day}} = \text{mg/L}$$

where:

NOAEL = no-observed-adverse-effect level.

LOAEL = lowest-observed-adverse-effect level.

Body weight = 70 kg for adult or 10 kg for child.

Drinking water volume = 2 L per day for adults or 1 L per day for children.

Uncertainty/Safety Factor = 10, 100 or 1,000.

Utilizing these equations, the following drinking water concentrations are developed for non-carcinogenic effects:

1. A one-day HA for 10-kg child.

2. A one-day HA for 70-kg adult.

3. A ten-day HA for 10-kg child.

4. A ten-day HA for 70-kg adult.

5. A lifetime AADI for a 70-kg adult.

The distinctions made between the HA calculations (items 1 through 4) are associated with the duration of anticipated exposure. Items 1 and 2 assume a single acute exposure to the chemical. Items 3 and 4 assume a limited period of exposure (possibly 1 to 2 weeks). The HA values will not be used in establishing a drinking water standard for the chemical. Rather, they will be used as informal scientific guidance to municipalities and other organizations when emergency spills or contamination situations occur. The AADI value (item 5) is intended to provide the scientific basis for establishing a drinking water standard based upon non-carcinogenic effects.

A NOAEL or LOAEL is determined from animal toxicity data or human effects data. For animal data, this level is divided by an uncertainty factor because there is no universally acceptable quantitative method to extrapolate from animals to humans. The possibility must be considered that humans are more sensitive to the toxic effects of chemicals than are animals. For human data, an uncertainty factor is also used to account for the heterogeneity of the human population in which persons exhibit differing sensitivity to toxic chemicals. An Office of Drinking Water (ODW) modification of the guidelines set forth by the National Academy of Sciences (NAS 1977, 1980) is typically used in establishing uncertainty factors as follows:

- An uncertainty factor of 10 is used when good acute or chronic human exposure data identifying a NOEL/NOAEL are available and are supported by acute or chronic toxicity data in other species.

- An uncertainty factor of 100 is used either when good acute or chronic toxicity data identifying a NOEL/NOAEL are available for one or more species (but human data are not available), or when good acute or chronic toxicity data identifying a LOAEL in humans are available.

- An uncertainty factor of 1,000 is used either when limited or incomplete acute or chronic toxicity data are available, or when good acute or chronic toxicity data identify a LOAEL (but not a NOEL/NOAEL) for one or more animal species.

The uncertainty factor used for a specific risk assessment is judgmental. Factors that cannot be incorporated in the NAS/ODW guidelines for selection of an uncertainty factor, but must be considered include: (1) the quality of the toxicology data, (2) the significance of the adverse effect and (3) the existence of counterbalancing beneficial effects.

If toxicological evidence requires the chemical to be classified as a potential carcinogen (CCl_4 is a known carcinogen in animals following ingestion), mathematical models are used to calculate the estimated excess cancer risks associated with the ingestion of the chemical via drinking water. The bioassay data used in these estimates are from animal experiments. In order

to predict the risk for humans, these data must be converted to an equivalent human dose. This conversion includes correction for non-continuous animal feeding, non-lifetime studies and for the difference in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average human body weight is 70 kg and that the average human consumes 2 liters of water per day. The multi-stage model is then fit to the equivalent human data to estimate the risk at low doses. The upper 95% confidence limit of this estimate is used. Excess cancer risks can also be estimated using other models such as the one-hit model, the Weibull model, the logit model and the probit model. There is no basis in the current understanding of the biological mechanisms involved in cancer to choose among these models. The estimates of low doses for these models can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of risk rate levels has an inherent uncertainty. This is because the tools of scientific measurement, by their very nature, involve both systematic and random error. In most cases, only studies using experimental animals have been performed. There is thus uncertainty when the data are extrapolated to humans. When developing risk rate levels, several other areas of uncertainty exist, such as (1) incomplete knowledge concerning the health effects of contaminants in drinking water, (2) the impact of test animal age, sex and species and the nature of target organ systems examined on the toxicity study results and (3) the actual rate of exposure of internal targets in test animals or humans. Dose-response data are usually only available for high

levels of exposure, not for the lower levels of exposure for which a standard is being set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

A. Noncarcinogenic Effects

1. Short-Term Exposure

Effects of acute exposure of rats to CCl_4 were reported by Korsrud et al. (1972). Groups of eight to nine Wistar rats (280 to 400 g) were administered single oral doses of CCl_4 (0 to 4,000 mg/kg) in corn oil. The rats were fasted before dosing and for 18 hours afterward, and then sacrificed. Liver weight and fat content, serum urea and arginine levels, and levels of nine enzymes in the serum were measured. In animals dosed with 40 mg/kg, liver fat, liver weight, serum urea, serum arginine, and levels of six of the nine enzymes were increased. At higher doses, the remaining three enzyme levels were also elevated. Histological examination of the liver revealed minimal evidence of change at the 20 mg/kg dose, while higher doses (40 mg/kg or above) resulted in loss of basophilic stippling, hydropic degeneration and occasional single cell necrosis. This study identified a dose of 20 mg CCl_4/kg as an acute LOAEL in rats.

Murphy and Malley (1969) investigated the effects of CCl_4 on corticosterone-inducible liver enzymes in rats. Groups of four to seven male

Holtzman rats (250 to 350 g) were administered 400, 800, 1,600, 2,400, or 3,200 mg undiluted CCl_4/kg by gavage. A single dose of 400 mg/kg or greater increased liver tyrosine-α-ketoglutarate transaminase and plasma alanine α-ketoglutarate transaminase, but increases were not observed in liver tryptophan pyrrolase or alkaline phosphatase within five hours.

Bruckner et al. (1984) investigated the short-term oral toxicity of CCl_4 in male Sprague-Dawley rats. Rats weighing 300 to 350 g were randomly divided into groups of five animals each. The animals were administered by gavage doses of 0, 20, 40, or 80 mg CCl_4/kg (in corn oil) daily for five days, allowed two days without dosing, and then dosed once daily for four additional days. One group of animals at each dosage level was sacrificed at 1, 4 or 11 days following the initiation of the dosing. In animals sacrificed after only one dose of 20 or 40 mg/kg, there were no significant[a] changes in blood urea nitrogen (BUN), glutamic-pyruvic transaminase (GPT) activity, sorbitol dehydrogenase (SDH) activity or ornithine-carbamyl transferase (OCT) activity, and no histopathological changes were observed in the liver or kidneys. At 80 mg/kg, small but statistically significant increases in GPT activity and BUN levels were observed. Furthermore, there was vacuolization of centrilobular cells adjacent to the central vein in each liver. In animals sacrificed after four daily doses, exposure to 20 mg/kg did not result in any significant alterations in enzymatic activities, but there was vacuolization of hepatocytes adjacent to the central vein. At doses of 40 and 80 mg/kg,

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[a]In the work of Bruckner et al. (1984), significance was assigned only for P values of 0.05 or less.

there were increases in SDH and GPT activities. Hepatic centrilobular vacuolization was noted in groups receiving 20 and 40 mg/kg. Midzonal vacuolization was observed in rats exposed to 80 mg/kg. In rats sacrificed after 11 days (nine doses), a dose of 20 mg/kg produced a significant increase in SDH and GPT activities, whereas doses of 40 or 80 mg/kg produced dose-dependent increases in serum levels of SDH, GPT and OCT. The extent of morphological changes in the liver was also dose-related. There was a progressive increase in serum enzyme levels and in hepatic lesion severity over the 11-day regimen in the 20, 40 and 80 mg/kg groups. This study identifies an acute (one-day exposure) NOAEL in rats of 40 mg/kg, and identifies a subacute (4- to 11-day exposure) LOAEL of 20 mg/kg.

In a similar experiment by Bruckner et al. (1984), male Sprague-Dawley rats (200 to 250 g) were randomly divided into groups of five animals each. The animals were administered by gavage doses of 0, 20, 80 or 160 mg CCl_4/kg (in corn oil) daily for five days, allowed two days without dosing, and then dosed once daily for four additional days. One group of animals at each dosage level was sacrificed 1, 4 or 11 days following the initiation of dosing. In animals sacrificed after one day, a dose of 20 mg/kg produced no significant changes in any parameter. Ingestion of 80 mg/kg produced significant increases in SDH and GPT, as well as hepatic centrilobular vacuolization. The 160 mg/kg dose produced marked increases in serum levels of all three enzymes and hepatic centrilobular necrosis. In animals sacrificed after four days, a dose-dependent liver injury was observed. With a dose of 20 mg/kg there were small, but statistically significant, increases in

SDH and GPT activities, as well as vacuolization of hepatocytes adjacent to the central vein of liver lobules. With doses of 80 and 160 mg/kg there were dose-dependent increases in serum levels of SDH, GPT and OCT. The livers of these animals showed midzonal vacuolization. In animals sacrificed after 11 days (nine doses), statistically significant increases in serum levels of all three enzymes were seen at all three dosage levels. In the 80 and 160 mg/kg groups, the increases were substantially greater at eleven days than at four days. Prominent hepatic necrosis was observed in the 80 and 160 mg/kg groups. This study identifies an acute (one-day exposure) NOAEL of 20 mg/kg in rats, and identifies a LOAEL of 20 mg/kg in subacute (4- to 11-day) exposure.

Many cases of human poisonings from CCl_4 ingestion and short-term inhalation exposure have been reported and compiled by NIOSH (1975) and USEPA (1983b). The liver and kidneys are the primary target organs of CCl_4 in humans. Unfortunately, most reports of human poisonings by CCl_4 are of limited utility in risk assessment, due to incomplete accounts, uncontrolled and unknown variables and individual differences in susceptibility to CCl_4.

The effects of oral administration of CCl_4 on the liver and kidneys of humans have been evaluated in condemned male prisoners in Ceylon (Docherty and Burgess 1922, Docherty and Nicholls 1923). Three of the prisoners were given 4 mL CCl_4 and two were given 5 mL CCl_4. Assuming a body weight of 70 kg, these doses correspond to 91 or 114 mg/kg. One prisoner was given 5 mL plus an additional 3 mL two weeks after the initial dose. The prisoners were executed 3 to 15 days after CCl_4 administration, and their liver and left

kidney removed at autopsy for microscopic examination. Although the kidneys of just two individuals exhibited alterations, the livers of four of the six showed fatty or granular degeneration. This study indicates that a dose of about 100 mg/kg is hepatotoxic in humans, but does not identify a NOAEL.

Stewart et al. (1961) evaluated the effects of exposure of human volunteers to CCl_4 vapor. Healthy males, 30 to 59 years of age, were exposed to concentrations of 63, 69 or 309 mg/m^3 of CCl_4 (99% pure) in an exposure chamber for 180 minutes at the two lower doses or 70 minutes at the highest dose. All subjects had undergone periodic physical examinations; some participated in more than one of the exposure experiments, which were conducted more than four weeks apart. Six subjects exposed to the highest concentration experienced no nausea or light-headedness, and CCl_4 was not detected in blood or urine during or after exposure. One of these six subjects had an increased level of urinary urobilinogen seven days after exposure. In addition, two of four subjects exposed to the highest concentration showed a decrease in serum iron within 48 hours after exposure. No CCl_4 was detected in the blood or urine of volunteers exposed at 63 or 69 mg/m^3, and the volunteers reported no effects. No changes in blood pressure, serum transaminase levels, or urinary urobilinogen levels were noted.

An epidemiologic study by Sonich et al. (1980) examined health effects of CCl_4 ingestion in humans. Seventy tons of CCl_4 were spilled in the Kanawha and Ohio Rivers in 1977. Measurements of raw water revealed maximum concentrations of 0.340 mg/L. Twenty-one cities situated along the river were

involved in the study. These cities represented areas that obtained their drinking water directly from the river and areas that obtained their drinking water from sources not influenced by the quality of the river water. By using river volumes and flow rates, periods of high exposure (1977) and low exposure (1976) to CCl_4 were estimated for each city along the river. The results of routine clinical tests measuring liver and kidney function along with basic epidemiologic information were abstracted from approximately 6,000 medical records. The results showed a positive and statistically significant ($P<0.05$) relationship between the CCl_4 exposure and the frequency of elevated levels of serum creatinine in exposed patients. Similar results were not found for the other parameters analyzed.

2. Long-Term Exposure

In a study by Bruckner et al. (1984), rats weighing 200 to 250 g were randomly divided into groups of 15 to 16 animals each. The animals were given by gavage 0, 1, 10, or 33 mg CCl_4/kg (in corn oil). The animals were dosed on a daily basis, five times weekly, for a total of 12 weeks. Blood samples were obtained from alternate animals at the following intervals: 2, 4, 6, 8, 10 and 12 weeks and 2 weeks post-treatment. The serum was analyzed for BUN, GPT, SDH and OCT. At a dose of 1 mg/kg/day, there were no significant clinical chemistry or histopathological changes at any time during the study. In rats receiving 10 mg/kg CCl_4/kg/day, SDH (the most sensitive index of hepatotoxicity) was elevated 2- to 3-fold over controls at most time-points during the study. These rats also exhibited mild hepatic centrilobular vacuolization. At a dose of 33 mg/kg/day, levels of GPT, SDH and OCT were markedly

increased, and severe hepatic lesions were apparent. Prominent fibrosis, bile duct hyperplasia, and hepatocellular vacuolization were seen in the portal and periportal regions of hepatic lobules. Nuclear pleomorphism, severe cytoplasmic degenerative changes, and hyperplastic nodules were commonly present in mid- and centrilobular areas. There was no evidence that CCl_4 was nephrotoxic. Serum enzyme levels returned to normal within the 2-week recovery period, but histopathological examination revealed that hepatic lesions were still present. Four of seven animals dosed with 10 mg/kg/day exhibited mild hepatic centrilobular vacuolization. The severity of the fibrosis and bile duct hyperplasia in animals dosed with 33 mg/kg/day was similar to that seen at 12 weeks. This subchronic study identifies a NOAEL of 1 mg/kg/day and a LOAEL of 10 mg/kg/day in rats.

In a chronic oral exposure study (Alumot et al. 1976), groups of 36 rats (18 males and 18 females, strain unspecified), were fed mash containing 0, 80, or 200 mg CCl_4/kg of feed. The authors calculated that animals on the 200 mg/kg diet ingested a dose of 10 to 18 mg CCl_4/kg/day. After two years, surviving animals were sacrificed. Serum concentrations of glucose, protein, albumin, globulin, urea, uric acid, cholesterol, GOT and GPT in the CCl_4-treated animals did not differ from those in controls. The authors stated that no fatty livers were detected in the treated animals, but no data were provided. Although no biochemical changes were attributed to CCl_4 exposure, interpretation of the results was complicated by the widespread incidence of chronic respiratory disease in the animals. All of the animals began to suffer from chronic respiratory disease after 14 months. By the end of the

experiment (24 months), more than half the animals were dead. Only three or four male rats survived at each dosage-level. Although the authors indicated that 10 to 18 mg/kg/day was an oral NOAEL of CCl_4 over two years, this conclusion may be questioned because the poor survival and chronic respiratory infection of experimental animals, and the apparent failure to examine organs for histopathological changes.

In a study by NCI (1976), groups of 50 Osborne-Mendel rats of each sex were administered CCl_4 in corn oil by gavage five times weekly for 78 weeks at two dosage levels: 47 or 94 mg/kg/day for males; 80 or 159 mg/kg/day for females. This dosage regimen resulted in approximately a 50% decrease in survival in all CCl_4-dosed groups. Bile duct proliferation, portal fibrosis, fatty degeneration and regenerative nodules were commonly seen in the livers of animals in each group. Since the lowest dose utilized (47 mg/kg/day in male rats) caused a significant decrease in survival and marked degenerative changes in the liver, it is not possible to establish a NOAEL or a useful LOAEL from this study.

B. Quantification of Noncarcinogenic Effects

1. One-Day Health Advisory

The acute animal study by Bruckner et al. (1984) has been selected to serve as the basis for the one-day Health Advisories in both the 10-kg child and the 70-kg adult, because this study clearly defined a one-day NOAEL

(40 mg/kg) and LOAEL (80 mg/kg) for CCl_4 based on changes in BUN, GPT, SDH and OCT and histopathological changes in the liver and kidneys of rats sacrificed 24 hours after dosing.

Korsrud et al. (1972) reported minimal morphological changes in some centrilobular hepatocytes in fasted rats given 20 mg CCl_4/kg orally. A dose of 40 mg/kg was required to produce significant changes in other parameters (i.e., increased liver fat, liver weight, serum urea and arginine, and serum enzyme levels). This study has not been selected because fasting increases the hepatotoxic effects of CCl_4 (Diaz Gomez et al. 1975, Harris and Anders 1980), but these data support the findings of Bruckner et al. (1984). The study by Sonich et al. (1980) has not been selected because only calculated estimates of CCl_4 ingestion were available, and because the effect noted (increased urinary creatinine levels) was not clearly adverse in its own right, and was not accompanied by any other adverse signs. The study by Murphy and Malley (1969) was not selected because only high doses were utilized and a NOAEL was not identified. The studies of Docherty and Burgess (1922) and Docherty and Nicholls (1923) were not selected, since no NOAEL was identified. The study by Stewart et al. (1961) has not been selected, since exposure was by inhalation.

Gastrointestinal absorption rates do not need to be considered in calculation of these HA values, since the NOAEL is based on effects observed following oral exposure.

The one-day HA for the 10-kg child is calculated as follows:

One-Day Health Advisory for Child

$$\frac{(40 \text{ mg/kg/day})\ (10 \text{ kg})}{(100)\ \ (1 \text{ L/day})} = 4.0 \text{ mg/L}$$

where:

40 mg/kg day = NOAEL, following one-day exposure in rats (Bruckner et al. 1984)

10 kg = assumed weight of child

1 L/day = assumed water consumption by a 10-kg child

100 = uncertainty factor. This factor was selected in accordance with ODW/NAS guidelines when a NOAEL from an animal study is employed.

One-day Health Advisory for Adult:

$$\frac{(40 \text{ mg/kg/day})\ (70 \text{ kg})}{(100)\ (2 \text{ L/day})} = 14 \text{ mg/L}$$

where:

40 mg/kg/day = NOAEL, following one-day exposure in rats (Bruckner et al. 1984).

70 kg = assumed weight of adult.

2 L/day = assumed water consumption by a 70-kg adult human.

100 = uncertainty factor. This uncertainty factor was selected in accordance with ODW/NAS guidelines when a NOAEL from an animal study is employed.

2. Ten-Day Health Advisory

The subchronic study by Bruckner et al. (1984) has been selected to serve as the basis for the ten-day HA for both the 10-kg child and the 70-kg adult. This study identified a LOAEL of 20 mg/kg/day in rats given 9 doses over 11 days, based on significant increases in serum enzyme levels and hepatic midzonal vacuolization by 11 days. Higher doses of CCl_4 caused even more extensive liver damage. No other studies of oral CCl_4 toxicity of appropriate duration for calculation of the ten-day HA values were located.

Gastrointestinal absorption rates do not need to be considered in calculation of these HA values, since the LOAEL is based on effects observed following oral exposure.

The 10-day HA for the 10 kg child is calculated as follows:

Ten-day Health Advisory for Child

$$\frac{(20 \text{ mg/kg/day})\ (10 \text{ kg})\ (9)}{(1{,}000)\ \ (1 \text{ L/day})\ (11)} = 0.16 \text{ mg/L } (160 \text{ µg/L})$$

where:

20 mg/kg/day = LOAEL, following 11-day exposure in rats (Bruckner et al. 1984).

9/11 = factor accounting for 9 doses given over 11 days.

10 kg = assumed weight of child

1 L/day = assumed water consumption by a 10-kg child.

1,000 = uncertainty factor. This factor was selected in accordance with ODW/NAS guidelines when a LOAEL from an animal study is employed.

The 10-day HA value for the 70-kg adult is calculated as follows:

Ten-day Health Advisory for Adult

$$\frac{(20 \text{ mg/kg/day})\ (70 \text{ kg})\ (9)}{(1{,}000)\ (2 \text{ L/day})\ (11)} = 0.57 \text{ mg/L } (570 \text{ µg/L})$$

where:

20 mg/kg/day = LOAEL, following 11-day exposure in rats (Bruckner et al. 1984).

9/11 = factor accounting for 9 doses given over 11 days.

70 kg = assumed weight of adult human

2 L/day = assumed water consumption by a 70-kg adult human

1,000 = uncertainty factor. This factor was selected in accordance with ODW/NAS guidelines when a LOAEL from an animal study is employed.

3. Adjusted Acceptable Daily Intake

The 12-week study by Bruckner et al. (1984) has been selected to serve as the basis for calculation of the ADI and AADI. Bruckner and co-workers dosed rats by gavage five times weekly for 12 weeks with doses of 0, 1, 10 or 33 mg

CCl_4/kg. This study identified a NOAEL of 1 mg/kg/day and a LOAEL of 10 mg/kg day.

The two-year study by Alumot et al. (1976), which identified an oral NOAEL of 10 to 18 mg/kg/day in rats, has not been selected because of the poor survival of both control and treated animals. The 78-week study by NCI (1976) has not been selected because it was not possible to discern a NOAEL or a meaningful LOAEL, as marked degenerative changes were seen in the liver of animals at all dosage levels.

Gastrointestinal absorption rates do not need to be considered in calculation of the ADI, since the NOAEL is based on effects observed following oral exposure.

The ADI for the 70-kg adult is calculated as follows:

ADI:

$$\frac{(1 \text{ mg/kg day})\ (70 \text{ kg})\ (5)}{(100)\ \ (10)\qquad\qquad (7)} = 0.050 \text{ mg/day } (50\ \mu\text{g/day}).$$

where:

1 mg/kg/day = NOAEL, following 90-day exposure in rats (Bruckner et al. 1984).

70 kg = assumed weight of adult.

100 = uncertainty factor. This uncertainty factor was selected in accordance with ODW/NAS guidelines in which a NOAEL from an animal study is employed.

10 = uncertainty factor. This uncertainty factor was selected because the length of exposure was only 90 days (as opposed to a lifetime).

5/7 = factor to account for dosing five days per week.

Using 50 µg/day as the ADI and assuming the 70-kg adult consumes two liters of drinking water per day, the AADI is calculated as follows:

AADI:

$$\frac{50 \text{ µg/day}}{2 \text{ L/day}} = 25 \text{ µg/L}$$

This AADI calculation assumes 100% of the human exposure derives from drinking water. This value may be modified upon the availability of relative source contribution data providing human exposure estimates from food, air and possibly the occupational environment.

C. Carcinogenic Effects

In a study by NCI (1976), groups of 50 Osborne-Mendel rats of each sex were administered CCl_4 in corn oil by gavage five times weekly for 78 weeks at two dose levels: 47 and 94 mg/kg/day for males, 80 and 160 mg/kg/day for females. The incidence of hepatocellular carcinomas was increased in rats

exposed to CCl_4 as compared with pooled colony controls. However, this increase was statistically significant only for females given the low dose as compared with the colony controls and not the matched controls. The absolute incidence of hepatic neoplasms was low (5% in the animals exposed to CCl_4). This may be attributed to the resistance by this rat strain to CCl_4 and to decreased survival time due to the toxicity of the chemical. The incidence of other neoplasms was acknowledged but not quantified. The results of this study were used by NAS (1978) in its carcinogenic risk estimate for CCl_4.

In a similar study (NCI 1976), B6C3F1 male and female mice (35 days of age, 50 per group) were given CCl_4 (1,250 or 2,500 mg/kg/day) in corn oil by gavage five times weekly for 78 weeks. Surviving mice were sacrificed at 92 weeks from the start of the study. There were 20 control mice of each sex that were given corn oil only. A necropsy and complete histological examination were performed on all mice. Most male and female mice treated with CCl_4 were dead by 78 weeks. Hepatocellular carcinomas were found in practically all mice receiving CCl_4, including those dying before termination of the test. The first carcinomas were observed in low dose female mice at 16 weeks, in high dose female mice at 19 weeks, in high dose males at 26 weeks and in low dose males at 48 weeks, compared to 72 weeks for colony control males and 90 weeks for colony control females. Cystic endometrial hyperplasia occurred in both control and treated female mice. Thrombosis of the atrium of the heart was seen in 9 of 41 high dose female mice (22%), all of which died with carcinomas of the liver. In summary, this study found CCl_4 to be highly hepatocarcinogenic in mice.

Edwards et al. (1942) investigated the carcinogenic potential of CCl_4 in mice. The mice used were inbred strain L with an extremely low incidence of spontaneous hepatomas. The test animals were 2.5 to 7.5 months of age at the onset of the experiment. The number of mice varied from 8 to 39 per group. Carbon tetrachloride was administered (0.1 mL of a 40% solution of CCl_4 in olive oil) by stomach tube two to three times weekly. Each dose thus consisted of 0.04 mL of CCl_4 per mouse, or approximately 2,500 mg CCl_4/kg (assuming an average body weight of 25 g). Mice were given 46 oral doses of CCl_4 over a 4-month period and were sacrificed and necropsied 3 to 3.5 months after the last treatment. Hepatomas developed in 34/73 mice (47%) given CCl_4. Hepatomas were observed in 7/15 younger male mice (47%), 21/39 older male mice (54%), 3/8 younger females (38%), and 3/11 older females (27%). Cirrhosis of the liver was not mentioned. One of 23 untreated (control) male mice (4%) and 0 of 28 females (0%), necropsied at 15 months of age, had tumors of the liver. Tumors were not present in 22 males and 28 females 18 months of age or in 27 female breeders 12-23 months of age. One of 24 male breeders (4%) had a tumor. In summary, strain L male and female mice were highly susceptible to the induction of hepatomas by CCl_4, and male mice were somewhat more susceptible than female mice.

Reuber and Glover (1970) studied the carcinogenicity of CCl_4 in five strains of rat: Japanese, Osborne-Mendel, Wistar, Black and Sprague-Dawley. Groups of 12 to 17 male rats of each strain were given twice weekly subcutaneous injections of CCl_4 (2,100 mg/kg) as a 50% solution in corn oil. Treated animals were sacrificed when moribund; controls for each strain were

sacrificed at the time as the last experimental animal. Lesions of the liver or other organs were not observed in control animals. The data suggested sensitivity to CCl_4-induced hepatic neoplasms varied widely among strains, but this may have been due in part to varying survival times among strains. All experimental animals of the Black rat strain were dead by 18 weeks, and those of the Sprague-Dawley strain by 16 weeks. The failure to find carcinomas in those strains may have been caused by an insufficient latency time. In the three other strains, lethality decreased in the same order that carcinogenicity increased.

Della Porta et al. (1961) evaluated the carcinogenicity of CCl_4 in Syrian golden hamsters. Ten female and ten male Syrian golden hamsters, 12 weeks old, were used. The animals were given CCl_4 (as a 5% solution in corn oil) by stomach tube once weekly. During the first seven weeks of the experiment, 0.25 mL of the solution containing 12.5 µL CCl_4 (about 200 mg CCl_4/kg) was given each week. Thereafter, the volume being given was reduced to 0.125 mL of solution containing 6.25 µL of CCl_4 (about 100 mg CCl_4/kg). After this treatment, the survivors were kept under observation for 25 additional weeks and then sacrificed. Detailed histopathological examinations were conducted on the survivors and on animals dying during the treatment period.

The body weights of the hamsters remained fairly constant during the 30 weeks of CCl_4 administration and increased slightly in the period following treatment. One female died at the tenth week of treatment. Three females and five males died or were sacrificed between the 17th and the 28th weeks. Three

females died at weeks 41, 43 and 54, respectively. The surviving three females and five males were sacrificed at the end of the 55th week.

The livers of hamsters dying during the treatment and at the 41st week exhibited cirrhosis and regenerative hyperplastic nodules. Cells in the nodules showed irregularities in shape, size and staining qualities of their cytoplasm and nucleus. Hepatic carcinomas were not found.

All of the animals dying or sacrificed 13 to 25 weeks after the end of the 30-week treatment had one or more liver cell carcinomas (a total of 22 tumors: 12 in the five females and 10 in the five males). Liver tissue not involved by the tumors showed cirrhotic changes. Hyperplastic nodules appeared during treatment, and carcinomas appeared after CCl_4 administration had been discontinued, which suggests that the nodules or benign tumors were precursor lesions for carcinomas.

In summary, Syrian golden hamsters appear to be sensitive to the carcinogenic effects of oral CCl_4. Although the number of animals in the study by Della Porta et al. (1961) was small, the authors considered the results to be significant because no hepatic tumors had been observed in historical controls (0/254) or in 30 females and 50 males given 0.5 mL corn oil by gavage twice weekly for 45 weeks. This study is the only report found in the literaure of CCl_4 induction of tumors in hamsters.

A summary of relevant literature on the health effects and cancer risk from CCl_4 for routes of exposure other than oral is contained in USEPA (1983b).

No reports of mutagenic activity associated with CCl_4 in any of the various Salmonella (Ames) assays were located. However, mutagenic activity associated with CCl_4 has been observed in a eukaryotic test system using the yeast Saccharomyces cerevisiae (Callen et al. 1980). This report has not been confirmed and should not be accepted as conclusive evidence of CCl_4 mutagenicity. Carbon tetrachloride did not cause chromatid gaps, deletions, or exchanges in cultured rat-liver cells exposed in vitro (Dean and Hodson-Walker 1979). Mirsalis and Butterworth (1980) found that treatment of male rats with CCl_4 (10 or 100 mg/kg administered by gavage) produced no increase in unscheduled DNA synthesis in primary cultures of rat hepatocytes. According to the authors, this observation indicates that CCl_4 does not act through a genotoxic mechanism.

The International Agency for Research on Cancer (IARC) has performed an assessment of the degree of evidence for the carcinogenicity of CCl_4 to humans and experimental animals (WHO 1982). This assessment concluded that CCl_4 is a Group 2B chemical (sufficient evidence for carcinogenicity in animals, but inadequate evidence for carcinogenicity in humans).

D. Quantification of Carcinogenic Risk

Data from the animal studies have been used by NAS (1977) and USEPA (1980a, 1983a, 1983b) to calculate the upper bound on the number of additional cancer cases that may occur when CCl_4 is consumed in drinking water over a 70-year lifetime. The results are shown in Table VIII-1.

Table VIII-1 Estimates of Additional Carcinogenic Risk Following Exposure to CCl_4 in Drinking Water[a]

Lifetime excess cancer risk	CCl_4 concentrations (µg/L)[b]		
	NAS (1977)[c]	USEPA (1980a)[d]	USEPA (1983b)[e]
10^{-4}	450	42.2	27.0
10^{-5}	45	4.2	2.7
10^{-6}	4.5	0.4	0.3

[a] Assuming an average daily drinking water consumption of two liters.
[b] Lower 95% confidence limit.
[c] Based on NCI (1976) (rats).
[d] Based on NCI (1976) (mice).
[e] Based on NCI (1976) (rats and mice), Edwards et al. (1942) (mice) and Della Porta et al. (1961) (hamsters).

The criteria for the USEPA and NAS risk calculations differ in two respects: (1) NAS used the multi-stage model, while USEPA used an "improved" multistage model; and (2) NAS used the data set from the National Cancer Institute (NCI 1976) study in male rats while USEPA used the data set from the NCI study in male mice (USEPA 1980a), or a geometric mean of four studies (NCI 1976 - mice; NCI 1976 - rats; Edwards et al. 1942 - mice; and Della Porta et al., 1961 - hamsters) (USEPA 1983b).

Ambient water quality criteria for CCl_4 calculated by USEPA (1980a) were based on increased lifetime cancer risk estimates of 10^{-5} (4.0 µg/L), 10^{-6} (0.40 µg/L), and 10^{-7} (0.04 µg/L). It is noteworthy that these estimates were derived by assuming a lifetime consumption of both drinking water (2 L/day) and aquatic life (6.5 g fish and shellfish/day) grown in waters containing the corresponding CCl_4 levels. Specifically, daily CCl_4 exposure assumptions were as follows: 94% from ingesting drinking water and 6% from consuming seafood "fish factor." The corresponding "drinking water only" concentrations are 4.4, 0.44, and 0.04 µg/L, respectively.

Using the same data set and a linear multistage model, WHO (1983) derived a recommended tentative limit for CCl_4 of 3 µg/L. This level should give rise to less than one additional cancer per 100,000 population (10^{-5}) for a lifetime of exposure assuming daily consumption of two liters of drinking water.

A unit risk estimate for humans of 0.37×10^{-5} for a person continuously exposed to 1 µg CCl_4 per liter of water was calculated by USEPA (1983a). This

corresponds to a risk of 1×10^{-6} at a concentration of 0.27 µg/L. Since no single study was judged to be entirely adequate for the risk assessment calculation, this estimate was based upon the geometric mean of the four animal studies discussed above. It should be noted that USEPA's Science Advisory Board approved this approach for calculating unit risk estimates for CCl_4, and this value is selected as the most appropriate estimate of the carcinogenic risk factor.

E. Existing Guidelines and Standards

In the absence of suitable data on the subacute oral toxicity of CCl_4, the USEPA and NAS have previously utilized alternate means to derive guidelines for short-term CCl_4 ingestion. The results of the initial year of the study by Alumot et al. (1976) were used by the Environmental Criteria and Assessment Office in Cincinnati (USEPA 1982) to derive the ambient water level of 3.3 mg CCl_4/L for protection against noncarcinogenic effects. Values of 2 L/day and 0.0065 kg/day for consumption of contaminated water and fish, respectively, were utilized in the calculation of this protective level. A bioconcentration factor of 18.75 L/kg for CCl_4 was also taken into consideration. A value of 3.5 mg/L was determined where CCl_4 exposure would be via drinking water only.

The USEPA (1981) and NAS (1980) have previously calculated SNARLS (Suggested No-Adverse-Response Levels) for CCl_4 in drinking water. These guidelines are summarized in Table VIII-2.

Table VIII-2 Summary of Existing Guidelines for CCl_4

	USEPA[a]	NAS[b]
One-day	0.2 mg/L	14 mg/L
Seven-day[c]	-	2 mg/L
Ten-day[c]	0.02 mg/L	-
Long-term	None[d]	None[e]

[a]USEPA (1981) used a LOAEL of 20 mg/kg (Korsrud et al. 1972) as the basis for their calculations.
[b]NAS (1980) used a LOAEL of 400 mg/kg (Murphy and Malley 1969) as the basis for their calculations.
[c]In the absence of subacute oral data, the NAS (1980) and USEPA (1981) calculated 7- and 10-day SNARLS by dividing their 1-day values by 7 and 10, respectively.
[d]The USEPA (1981) did not calculate a long-term SNARL due to a lack at the time of acceptable chronic oral exposure data.
[e]The NAS (1980) did not determine a long-term SNARL because of NAS policy at that time not to calculate such values for animal carcinogens.

F. Special Considerations

The toxicity of CCl_4 is largely dependent upon its metabolism by enzyme systems in the liver and elsewhere. The activity of these enzyme systems may be increased (induced) by exposure to a variety of chemicals, and this increased metabolic activity may lead to increased toxicity of CCl_4. This phenomenon has been reported for compounds such as ethanol (Cornish and Adefuin, 1966), secondary alcohols and ketones (Traiger and Plaa 1971, Traiger and Bruckner 1976), PCBs and PBBs (Kluwe et al. 1979), phenobarbital (Cornish et al. 1973) and chlordecone (Curtis et al. 1979). Alcohol ingestion in particular has been associated with increased susceptibility of humans to CCl_4 hepatotoxicity and nephrotoxicity (USEPA 1983b). Smoking, consumption of charcoal-broiled beef and ingestion of certain drugs (e.g. phenobarbital) are factors known to significantly induce microsomal enzyme activities in humans, thereby potentially creating greater risk than normal.

G. Summary

The recommended one-day and ten-day HA values for both children and adults, the one-day and ten-day AADI, and the estimated excess cancer risks are summarized in Table VIII-3.

Table VIII-3 Summary of Quantification of Toxicological Effects of CCl_4

	Drinking water concentration (mg/L)		
	10-kg Child	70-kg Adult	Reference[a]
One-Day Health Advisory	4.0	14	Bruckner et al. (1984)
Ten-Day Health Advisory	0.16	0.57	Bruckner et al. (1984)
Lifetime AADI	--[b]	0.025	Bruckner et al. (1984)
Excess cancer risk			
10^{-4}		27 µg/L	USEPA (1983b)
10^{-5}		2.7 µg/L	USEPA (1983b)
10^{-6}		0.3 µg/L	USEPA (1983b)

[a]Reference for NOAEL or LOAEL used in calculating HA or AADI values.
[b]Not calculated because the AADI is based on lifetime exposure.

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